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Frog Embryo Teratogenesis Assay: *Xenopus* (FETAX) is a 96-hour, whole-embryo bioassay designed to detect potential developmental toxicants. The primary goal of this research was to improve the predictability and increase the overall utility of FETAX as a screen for developmental toxicants that pose a hazard to human health. The first specific aim was to explore the use of phenobarbital, \(\beta\)-naphthoflavone, and isoniazid-induced rat liver microsomes as an exogenous metabolic activation system. This particular inducing system supplanted the presently-used Aroclor 1254 system which was recently proven to be somewhat unreliable. Post-isolation mixtures of these microsomes represented a broader spectrum of P-450 isozymes, and thus, bioactivated/inactivated a wider range of compounds. The second specific object was to develop and evaluate a standardized metabolic activation system test kit for FETAX which could be used by other FETAX users, including the U.S. Army. The findings from this research demonstrated that the post-isolation mixed microsomes were highly effective and that the test kits provided a reliable standardized means of routinely using the exogenous activation system with FETAX.

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#### **FOREWORD**

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#### Introduction

The vast majority of the epidemiological evidence acquired today suggests that most birth defects are the result of non-genetic environmental factors. Approximately 100,000-150,000 chemicals are currently available in the market place with 2,500 new chemicals released each year. Prior to their release into the market place and ultimately the environment, the safety of these compounds must be firmly established. The expense and time-consuming nature of conventional testing has warranted the development, validation, and eventual wide-spread use of alternative, nonmammalian model systems. FETAX (Frog Embryo Teratogenesis Assay - Xenopus) is a 4-day, non-mammalian, whole-embryo bioassay designed to evaluate potential teratogenic hazard by: 1) direct chemical/complex environmental mixture screening, and 2) use as a tool to evaluate toxicological mechanisms of action. FETAX has been extensively validated as a screening assay using both pure chemical compounds and complex environmental mixtures (wastewater effluent, sediments, and groundwater). Because *Xenopus* lack many metabolic enzyme systems including the mixed-function oxidase (MFO) system through the first 96-hours of development, an exogenous metabolic activation system (MAS) was developed (1) and evaluated (2-7) using Aroclor 1254-induced rat liver microsomes. However, the use of alternative, more advantageous inducing agents with the rat liver microsomes have only recently been explored with the FETAX assay.

Of approximately three million infants born alive each year, 13.3 per 1000 die within the first year (8). Approximately 2 to 3 percent of infants born alive have major congenital malformations recognized within the first year of life (9). When defects that only become apparent later in life are included, the frequency of major and minor malformations increases to about 16 percent (10). Approximately 7 percent of newborns are born prematurely (before the 37th week) and 7 percent of infants born at full term have low birth weights (2.5 kg or less) (11).

The NIOSH Registry of Toxic Substances (12) indicated that of some 38,000 chemicals in common industrial use, only 1,930 of these had been tested for teratogenecity with 580 demonstrating positive effects. In the Catalog of Teratogenic Agents complied by Thomas Shepard (13), over 600 chemical agents are presented with only 20 known to induce birth defects in humans. Obviously many gaps exist in our knowledge of how to predict which chemicals, among the million present in our environment or workplace, possess teratogenic potential; and the correlation between results in animal studies and effects in humans.

Developing an exogenous MAS for FETAX which is not dependent on Aroclor 1254 induction is imperative if FETAX is to be widely effective and universally accepted as a surrogate developmental toxicity screening assay. The use of Aroclor 1254 as an inducing agent presents several problems including: 1) inconsistent induction due to congener concentration variability from lot to lot, 2) isozyme repression (CYP2E1), 3) not readily available due to reduction in the use of PCBs, and 4) hazardous waste and human health hazard issues. Thus, developing postisolation mixtures of rat liver microsomes induced by β-naphthoflavone (CYP1A1 and 1A2), phenobarbital (CYP2A6/2B6), and isoniazid (CYP2E1) will help alleviate the problems associated with the use of Aroclor 1254 while providing a broad-spectrum of induced isozymes. The

previous mentioned isozymes represent the primary P-450 isozymes responsible for Phase I biotransformation of potential developmental toxicants. Additional inducing agents may be added to this repertoire as needed in the future.

By evaluating FETAX as a developmental toxicity screening system, the U.S. Army, scientific community, chemical and pharmaceutical producing industries, appropriate regulatory agencies, and ultimately the public will be provided with a versatile alternative, short-term prescreening assay and mechanistic model for the commonly used three segment test protocol. As opposed to many other advance technologies this bioassay can be developed, validated, and utilized quickly.

The Xenopus test system is a non-mammalian, alternative assay which offers some short and longterm solutions to current problems associated with developmental hazard evaluation. The problems are: increasing regulatory requirements, test cost, test duration, and the reduction in the use of mammals in research. Existing in vivo mammalian test systems are effective for the testing of drugs and cosmetics, but are too lengthy, expensive and technically difficult for evaluating mechanisms of action. In addition, the number of chemicals in production which require testing is increasing at a rate faster than is feasible to handle with in vivo test systems. These events have produced a climate which favors the use of alternative test systems for eventual refinement and possible reduction of in vivo assays. In a study conducted for NIH, the National Academy of Sciences recently concluded that the development and use of lower life form models for toxicity of testing should be emphasized. Tighter restrictions will limit the use of in vivo assays and increase the costs associated with their performance. The Office of Technology assessment for the United States Congress has recently completed an assessment of the use of animals in testing, research and education. Congressional options emanating from this study include support for the further development and use of *in vitro* test systems when appropriate. Thus, cost-effective, predictive developmentally relevant, tests are urgently needed to allow the rapid evaluation of developmental toxicants in the environment and to elucidate toxicological mechanisms of teratogenesis.

The ultimate goal of the proposed research is to protect humans from developmentally toxic chemicals, regardless of where exposure may take place, by either direct aversion or by increasing the understanding of toxicological mechanisms of teratogenesis. Knowledge of toxicological mechanisms of actions of commonly used drugs by physicians and pharmacists should increase confidence in the administrations of these drugs during pregnancy. In addition, this information will allow chemists and biologists to make educated, well-informed decisions on the development and manufacture of chemicals by understanding potential toxicological effects.

The specific goal of the proposed research was to improve the performance of FETAX by developing a more efficacious MAS using mixtures of \( \beta \)-naphthoflavone-, phenobarbital, and isoniazid-induced rat live microsomes. Recent discussions with chemical and pharmaceutical manufacturing industries, as well as, the National Institute of Environmental Health Sciences (NIEHS), have indicated that these groups are interested in the FETAX system, but would not use or recommend the system on a large-scale basis unless the model could both screen for human developmental toxicants and elucidate mechanisms of action by including a standardized testing protocol with an effective MAS. Furthermore, the groups indicated that the screening assay must

be well developed and properly validated. Therefore, the development and proper validation of an exogenous MAS for FETAX must be performed before this assay can be successfully utilized.

The original work on developing a developmental toxicity screening assay using *Xenopus* embryos to detect environmental teratogens was performed in the laboratories of Greenhouse (14) and Dumont (15). Greenhouse used 48-hour exposures to military compounds (N-phenyl-αnaphthylamine and various hydrazines) to demonstrate toxic and teratogenic effects on developing embryos. Early studies in our laboratory have demonstrated that FETAX can be used with a variety of chemicals and complex mixtures. The endpoints included: LC50 (mortality), EC50 (malformation-teratogenesis), no observed effect concentration (NOEC), minimum concentration to inhibit growth (MCIG) [both length and developmental stage], motor behavior, pigmentation, and gross anatomy. A Teratogenic Index (TI), the ratio of the 96-h LC50/96-h EC50 (malformation) was developed and has been successfully used as a measure of the relative developmental hazard of a substance (1-7, 16-20). Thus, assessment of teratogenic potential has been based on TI values, embryo growth, and types and severity of induced malformations. Generally, TI values <1.5 have indicated low teratogenic potential, whereas higher values have signified an increase in the potential teratogenic hazard, as there was little or no separation between the concentrations that cause embryo malformation, but cause no embryolethal effects, and concentrations which were embryolethal. Greater TI values signified a larger separation between the two responses, and thus, a greater possibility of embryos being malformed in the absence of significant embryo lethality. Types and severity of induced malformations have also been considered, especially for compounds with TI values <1.5 which produce serious defects of major organ systems. Such compounds may still pose a serious threat, possibly as embryolethal agents. We are currently establishing a data base to assess the utility of the TI value in assessing teratogenic hazard. Test chemical exposures have been continuous for 96-hours. Mortality and stage of development were checked at hours 24, 48, 72, and 96, while the other endpoints were recorded only at hour 96. Test compounds renewal was performed daily throughout the tests. Data collection was simple, as all observations were made with a dissection microscope. The data collected using FETAX have been in harmony with the criteria for an in vitro teratogenesis screen suggested by Kimmel et al. (21). These criteria included: good concentration-response relationships, adequate number of embryos, and easily defined and quantified endpoints.

In addition, an American Society of Testing and Materials (ASTM) New Standard Guide for the Conduct of FETAX has been recently published (22). An "Atlas of Abnormalities" companion document to the ASTM guide has been produced in order to aid in the proper scoring of malformations (23).

#### **FETAX Test Performance**

Dumont (unpublished) has accumulated validation data on 45 compounds with nearly 85 percent correspondence to mammalian results. Sabourin (24) have completed testing of 32 compounds with 83% predictive accuracy. However, neither of these two investigators used the exogenous MAS developed for FETAX. With over 100 compounds tested, we have approached 95% predictive accuracy in our laboratory using the Aroclor 1254-induced exogenous MAS.

Correlation between laboratories has been encouraging, as well. For example, Courchesne and Bantle (25) found a teratogenic index for hydroxyurea of 4.3, whereas Sabourin recorded 4.5 for the same chemical. We have performed an extensive interlaboratory validation study with several laboratories across the United States (26-29). Results obtained were extremely encouraging and warranted further development and validation of a versatile, yet more robust MAS for FETAX.

Sabourin and Carlton (unpublished) determined that the same stock of diphenylhydantoin caused pericardial edema as the primary anomaly in both the cultured whole rat embryo and *Xenopus* embryos. Dumont et al. (30) similarly found that meclizine induced hydrocephalia in both frogs and mammals and that other teratogens produced similar abnormalities in both frogs and mammals (developmental mimicry). Courchesne and Bantle (25) reported that a number of genotoxic chemicals caused the same general types of malformations in both *Xenopus* and rodent embryos. Dawson et al. (16) have developed an artificial medium (FETAX solution) and have carried out a preliminary validation using five compounds ranging from a nonteratogen to a strong teratogen producing results corresponding to current mammalian literature. Fort et al. (1,3-7) have found that twenty-six known mammalian teratogens tested with FETAX produced similar malformations as observed in mammals.

#### **Evaluation of Exogenous Metabolic Activation System**

As mentioned in previous sections, the inability of young *Xenopus* embryos to metabolize xenobiotics prompted the development of an exogenous MAS. We developed the MAS by using Aroclor 1254-induced rat liver microsomes and an NADPH generating system co-cultured with the embryos. The extensively studied proteratogenic compound, cyclophosphamide (31), was used in the initial pilot study to assess the feasibility of the system (1). Results indicated that cyclophosphamide was bioactivated into an embryotoxic form using the exogenous MAS. Also, addition of the microsomes, antibiotics (used to combat bacterial contamination) and a NADPH generating system did not increase mortality and malformation rates above FETAX solution control values. Specifically, activation reduced the 96-hour LC50 of cyclophosphamide 5.7-fold from 8.0 to 1.4 mg/mL. The 96-hour EC50 (malformation) was reduced 15.5-fold from 6.2 to 0.4 mg/mL. The 96-hour TI was increased 2.7-fold upon activation from 1.3 to 3.5. The severity of the malformations were used in FETAX compared to uninduced rat liver microsomes and generating system (32).

Additional validation studies were performed with Aroclor 1254-induced microsomes co-cultured with *Xenopus* embryos (3). Rifampicin, 2-acetylaminofluorene, and benzo(α)pyrene were all bioactivated as expected, while cytochalasin D was inactivated. The inclusion of the MAS had no effect on the developmental toxicity of zinc as expected. Nicotine had a TI value of 325 when tested in FETAX without Aroclor 1254-induced rat liver microsomes (2). This value was reduced to 3.5 when the embryos were co-cultured with an Aroclor 1254-induced MAS. However, bioactivation did not always result in a change in the TI value. Bantle et al. (33) found that metabolic activation reduced the 96-hour LC50 and EC50 (malformation) of N-ethyl-N-nitrosourea by a factor of 73, but did not change the TI value.

#### **Evaluating Toxicological Mechanisms of Action**

Preliminary studies have demonstrated the utility and versatility of FETAX for evaluating the toxicological mechanisms of action of diphenylhydantoin (4). In the initial study, teratogenic potentials of diphenylhydantoin (DPH) and hydroxylated metabolites (HPPH) were evaluated with FETAX. Xenopus embryos were exposed to DPH and HPPH in two separate static-renewal experiments with and without the presence of an exogenous MAS for 96 hours. Two separate concentration-response tests were also conducted with DPH and HPPH with a MAS modulated by various MFO inhibitors [carbon monoxide (CO) (broad-spectrum cytochrome P-450), cimetidine (mainly cytochrome P-450), ellipticine (cytochrome P-4490], and an epoxide hydrolase inhibitor (cyclohexene oxide). Assessment of the potential teratogenic hazard was based on TI values, types and severity of malformations, and embryos growth endpoints. Addition of the intact MAS to DPH increased the 96-hour LC50 and EC50 (malformation) from approximately 74.5 and 32.4 mg/L to 126.4 and 62.4 mg/L, respectively. The TI was reduced 1.2-fold. Both p-HPPH and m-HPPH were much less developmentally toxic than DPH. CO and cimetidine inhibition of cytochrome P-450 maintained much of the developmentally toxicity of DPH, whereas ellipticine inhibition of cytochrome P-448 was much less effective in maintaining the developmental toxicity of DPH. Cyclohexene oxide inhibition of epoxide hydrolase markedly increased DPH-induced embryotoxicity decreasing the 96-hour LC50 from approximately 74.5 to 38.6 mg/L. These results suggested that unmetabolized DPH and an embryotoxic epoxide intermediate served as the teratogenic species in FETAX.

In a separate study, the developmental toxicity of isoniazid (INH) and the metabolites acetylhydrazide (AH) and isonicotinic acid (INA) were examined with FETAX (5). Late *Xenopus* blastulae were exposed to INH, AH, and INA for 96-hours in two separate static-renewal tests with and without the presence of three differently induced metabolic activation systems (MAS). The MAS consisted of uninduced, Aroclor 1254-induced, and INH-induced rat liver microsomes. Addition of the INH-induced MAS decreased the 96-hour LC50 of INH and AH approximately 1.6-fold and 7.0-fold, respectively. The 96-hour EC50 (malformation) of INH was virtually unaffected; however, the INH-MAS decreased the TI value nearly 1.8-fold. The 96-hour EC50 (malformation) of AH increased approximately 2.0-fold, decreasing the TI value 15.9-fold. INA yielded a TI value of 2.5. Neither the uninduced MAS nor the Aroclor 1254-induced MAS had an affect on any of the compounds tested and none of the MASs affected the developmental toxicity of INA. Results from this study suggested that MFO metabolism may have altered the developmental toxicity of INH *in vitro* by producing a more embryolethal, but less teratogenic metabolite(s) than INH or AH themselves.

Finally, potential mechanisms of acetaminophen-induced developmental toxicity were evaluated using FETAX (7). Early *Xenopus* embryos were exposed to acetaminophen for 96-hours in two definitive concentrations-response assays with and without an exogenous MAS. Two static-renewal tests with acetaminophen and the MAS treated with carbon monoxide, cimetidine, ellipticine, diethyl maleate, and supplemented with glutathione were also performed. Addition of the MAS decreased the 96-hour LC50 and EC50 (malformation) values of unactivated acetaminophen 3.9-fold and 7.1-fold, respectively. Addition of the carbon monoxide- and ellipticine-inhibited MAS, as well as, the glutathione-supplemented MAS decreased the

developmental toxicity of activated acetaminophen to levels near that of the unactivated parent compound. Cimetidine-inhibited MAS also reduced the developmental toxicity of acetaminophen, but not to the extent observed with the carbon monoxide- and ellipticine-inhibited, or glutathione-supplemented MAS Addition of the diethyl maleate-treated MAS substantially increased the developmental toxicity of acetaminophen. Results indicated that a highly reactive intermediate formed as the result of mixed function oxidase-mediated metabolism (possibly P-448) significantly increased the developmental toxicity of acetaminophen. Glutathione was also found to play a major role in intermediate detoxification *in vitro*.

In summary, the developmental toxicity of four test compounds (cyclophosphamide, coumarin, 2-acetylaminofluorene [2-AAF], and trichloroethylene) was evaluated using the traditional static-renewal method described in the ASTM Standard Guide for conducing FETAX (22) with minor modification. Differently-induced rat liver microsomes in the form of a test kit was co-cultured with FETAX in an identical fashion to that described in Experimental Methods Section. Results from these studies are provided in this report.

## **Body**

#### **Experimental Methods**

#### **Animal Care and Breeding**

*Xenopus* adult care, breeding, and embryo collection were performed as described in the ASTM Standard Guide (22).

#### **Metabolic Activation System(s)**

The metabolic activation system consisted of post-isolation mixed sets of \( \mathbb{B}\)-naphthoflavone, phenobarbital, and isoniazid-induced rat liver microsomes. Several studies including some in our own laboratories have demonstrated that the use of Aroclor 1254-induced microsomes with compounds metabolized by Aroclor 1254-repressed isozymes (pyrazole/isoniazid-induced P-450) was counter-productive, thus isoniazid was also been selected as a P-450 inducing agent. In addition, several chemicals have been shown to be metabolized by Aroclor 1254-repressible isozymes including: trichloroethylene, acetone, ethanol, and N-nitrosodimethylamine. Since the use of commercial preparations of rat liver microsomes, including both S-9 and lypholyzed purified preparations, has been unsuccessful due to a combination of high residual Aroclor 1254 levels and lipid peroxidation-induced toxicity, we purified rat liver S-9 supernatant. Purification of the rat liver microsomes was performed by the method of Kitchin and Woods (34). The pelleted microsomes were resuspended in cold 0.05 M Tris-HCl buffer and the protein content was determined. For each experiment performed the same amount of P-450 activity was added to the dishes even though the amount of protein varied. P-450 activity inferred by the amount of formaldehyde generated from the N-demethylation of aminopyrine and N-nitrosodimethylamine by the method of Nash (35). After quantification, the microsomes were split into four aliquots and

frozen in liquid nitrogen for use in each succeeding day of the experiment. Freezing of microsomes was determined to be an acceptable procedure (36). Randomly selected aliquots of the differently induced microsome mixes were treated with CO immediately prior to co-culture with the *Xenopus* embryos.

#### **Assay Protocol**

All FETAX tests were conducted in accordance with the methods described in the ASTM Standard Guide (22) with the minor modifications described below. For test conducted with unactivated toxicant (no MAS), groups of 20 embryos were placed in 60 mm plastic Petri dishes with varying concentrations of the toxicant. The test compounds were dissolved in FETAX solution, a reconstituted water media designed for the culture of *Xenopus* larvae (16). One percent DMSO (v/v) was used as a co-solvent as needed (4). Four separate dishes of 20 embryos were exposed to FETAX Solution alone and designated FETAX Solution controls. For each definitive test, 12-18 concentrations were tested in duplicate. Tests conducted with the MAS and the inhibited MAS were also conducted in duplicate with 20 embryos per replicate concentration. Each activated treatment received 0.04 U/ml of APD activity (expressed as μM formaldehyde generated/minute), a NADPH generating system, and 100 U/mL penicillin--100 U/mL streptomycin. The NADPHH generating system will consisted of 3.5 mM glucose-6-phosphate, 0.31 IU glucose-6-phosphate dehydrogenase, 0.1 mM NADP, and 0.7 μM NADPH. Controls for the MAS, CO inhibited-MAS, cyclophosphamide [FETAX reference proteratogen] (2), and unactivated toxicant were also be conducted with each individual test.

One range test, one definitive unactivated test, and two separate definitive tests with and without the MAS were conducted with each compound in the same format utilized during Phase III of the recent interlaboratory study (ILS) conducted with FETAX (29). Although limited testing was performed with the Aroclor 1254-induced MAS, previous ILS work (26-29) suggested that the most successful approach for consistent interlaboratory testing was to perform testing in the following manner: 1) initial range test to estimate 96-hour LC50 and EC50 (malformation) values, 2) definitive unactivated test (no MAS) using sliding scale to select concentrations (29), and 3) two definitive concentration-response experience tests with and without the exogenous MAS using the predicted EC5 (malformation) and LC5, EC16 and LC16, EC50 and LC50, EC84 and LC84, and EC95 and LC95 from the definitive unactivated test. This approach has been highly effective in testing performed since the completion of the ILS program (26-29). The pH of all stock solutions was strictly maintained between 7.0-7.2 if possible. Embryos were cultured at 23±1°C throughout the test. All solutions will be removed every 24 hours of the 4-day test and fresh solutions will be added. Dead embryos were removed when solutions were changed. Following 96-hours of exposure, surviving embryos were fixed in 3.0% formalin, pH 7.0. The number of live-malformed embryos and the stage of development (37) were then be ascertained using a dissection microscope.

#### **Data Analysis**

Probit analysis (38) was used to determine the 96-hour median lethal concentration (96-hour LC50) the 96-hour EC50 (the concentration inducing gross terata in 50% of the surviving larvae) and their respective 95% fiducial intervals. Comparison of concentration-response data will be performed by the method of Sprague and Fogels (39). TI values (18) were determined for each test. Head to tail length will be measured as an indicator of embryo growth using Sigma Scan® digitizing software (Jandel Scientific, Corte Madera, CA) and a personal computer. Concentrations inducing growth inhibition (MCIG) were calculated using the Williams test (grouped, P=0.05).

#### **Results and Discussion**

#### **Development of Exogenous Metabolic Activation System**

Preliminary range finding results of tests with cyclophosphamide, coumarin, 2-AAF, and trichloroethylene, are provided in Table 1. Results from these unactivated definitive tests were used to generate the concentrations to be used in the evaluation of the differently induced MASs. As previously mentioned in the Experimental Methods section, tests with each of the four compounds with and without each of an Aroclor 1254-, phenobarbital-, \(\beta\)-naphthoflavone-, and isoniazid-induced MAS were performed concurrently. In addition, the use of post-isolation mixes of the phenobarbital-, B-naphthoflavone-, and isoniazid-induced MASs were prepared and tested concurrently with the other treatments.

Results of MAS performance evaluation with cyclophosphamide is provided in Table 2. Unactivated cyclophosphamide or cyclophosphamide co-cultured with the isoniazid-induced MAS were not developmentally toxic at concentrations >4,000 mg/L. In Test No. 1, Aroclor 1254induced MAS reduced the 96-hour LC50 and EC50 (malformation) values to 1,500.0 and 590.0 mg/L, respectively. Phenobarbital-induced MAS reduced the 96-hour LC50 and EC50 (malformation) values to 1,700.0 and 610.0 mg/L, respectively. The \(\beta\)-naphthoflavone-induced MAS less effectively bioactivated cyclophosphamide to 96-hour LC50 and EC50 (malformation) values of 3,090.0 and 1,810.0 mg/L, respectively. As previously mentioned, the isoniazid-induced MAS was ineffective in bioactivating cyclophosphamide. The post-isolation mixture of the phenobarbital-, \u03b3-naphthoflavone-, and isoniazid-induced MASs was highly effective in bioactivating cyclophosphamide producing LC50 and EC50 (malformation) values of 1,340.0 and 400.0 mg/L, respectively. The mixed MAS also produced the greatest TI value of 3.4 and inhibited growth at comparable levels (100.0 mg/L) to the Aroclor 1254- and phenobarbitalinduced MAS tests. Results from Test No. 2 were similar to results from Test No. 1 in which each of the Aroclor 1254-induced, phenobarbital-induced, and mixed MASs were successful in bioactivating cyclophosphamide, whereas, the \( \beta\)-naphthoflavone- and isoniazid-induced systems were less effective and ineffective in bioactivating cyclophosphamide, respectively. Bioactivated cyclophosphamide induced microencephaly, ophthalmic abnormalities, and severe skeletal kinking. These abnormalities were similar to those recorded in previous studies and similar to those identified in rodents, rabbits, and monkeys (1, 31). The results of the present study were not necessarily unexpected as cyclophosphamide has been shown to primarily bioactivated by

Table 1 **Results of Unactivated Definitive FETAX Tests** 

Compound	LC50 <sup>1</sup>	EC50 <sup>1,2</sup>	TT	MCIG <sup>4</sup>
Cyclophosphamide	6760.0 (6650.0 - 6880.0)	4830.0 (4710.0 - 4950.0)	1.4	6000.0
Trichloroethylene	490.0 (480.0 - 500.0)	51.0 (49.0 - 53.0)	9.6	400.0
Coumarin	168.7 (154.2 - 186.2)	59.8 (58.5 - 61.1)	2.8	100.0
2-Acetylaminofluorene	110.0 (105.0 - 115.0)	30.0 (25.0 - 35.0)	3.7	90.0

<sup>&</sup>lt;sup>1</sup> Expressed as mg/L.

<sup>2</sup> Malformation.

<sup>3</sup> Teratogenic Index calculated by dividing the 4-d LC50/4-d EC50 (malformation).

<sup>4</sup> Minimum Concentration to Inhibit Growth (Williams Test, P=0.05).

Table 2

Results of FETAX tests with Cyclophosphamide and Differently-Induced
Exogenous Metabolic Activation Systems (MASs)

Test No.	MAS	$LC50^{1}$	EC50 <sup>1, 2</sup>	$TI^3$	MCIG <sup>4</sup>
1	None	>4000.0 (ND)	>4000.0 (ND)	ND	>4000.0
	Aroclor 1254	1500.0 (1420.0-1580.0)	590.0 (510.0-680.0)	2.5	100.0
	Phenobarbital	1700.0 (1610.0-1810.0)	610.0 (530.0-700.0)	2.8	100.0
	β-Naphthoflavone	3090.0 (3000.0-3170.0)	1810.0 (1720.0-1910.0)	1.7	1000.0
	Isoniazid	>4000.0 (ND)	>4000.0 (ND)	ND	>4000.0
	Mixed <sup>5</sup>	1340.0 (1260.0-1430.0)	400.0 (340.0-470.0)	3.4	100.0

(continued)

# Results of FETAX tests with Cyclophosphamide and Differently-Induced **Exogenous Metabolic Activation Systems (MASs)**

(continued)

Test No.	MAS	LC50 <sup>1</sup>	EC50 <sup>1, 2</sup>	TI <sup>3</sup>	MCIG <sup>4</sup>
2	None	>4000.0 (ND)	>4000.0 (ND)	ND	>4000.0
	Aroclor 1254	1850.0 (1740.0-1980.0)	710.0 (620.0-800.0)	2.6	100.0
	Phenobarbital	1590.0 (1510.0-1670.0)	920.0 (870.0-970.0)	1.7	100.0
	β- Naphthoflavone	3130.0 (3000.0-3280.0)	1860.0 (1750.0-1970.0)	1.7	1000.0
	Isoniazid	>4000.0 (ND)	>4000.0 (ND)	ND	>4000.0
	Mixed <sup>5</sup>	1690.0 (1580.0-1810.0)	720.0 (630.0-810.0)	2.4	100.0

<sup>1</sup> Expressed as mg/L.

<sup>&</sup>lt;sup>2</sup> Malformation.

Teratogenic Index calculated by dividing the 4-d LC50/4-d EC50 (malformation).

Minimum Concentration to Inhibit Growth (Williams Test, P=0.05).

<sup>&</sup>lt;sup>5</sup> Consists of equal post-isolation activity mix of phenobarbital-, β-naphthoflavone-, and isoniazid-induced rat liver microsomes.

phenobarbital-inducible P-450, but with some cross specificity with aryl hydrocarbon hydroxylase isozymes (B-naphthoflavone-inducible P-450) (1,29).

Results of MAS performance evaluation with coumarin is provided in Table 3. Tests with unactivated coumarin produced 96-hour LC50 and EC50 (malformation), TI, and MCIG values of 129.4 mg/L, 49.0 mg/L, 2.6, and 50.0 mg/L. In Test No. 1, Aroclor 1254-induced MAS reduced the 96-hour LC50 and EC50 (malformation) values to 68.9 and 25.9 mg/L, respectively. Phenobarbital-induced MAS reduced the 96-hour LC50 and EC50 (malformation) values to a lesser extent to 93.3 and 51.5 mg/L, respectively. The β-naphthoflavone-induced MAS effectively bioactivated coumarin to 96-hour LC50 and EC50 (malformation) values of 57.2 and 16.9 mg/L, respectively. The isoniazid-induced MAS was also less effective in bioactivating coumarin producing LC50 and EC50 (malformation) values of 98.0 and 46.1 mg/L. The postisolation mixture of the phenobarbital-, B-naphthoflavone-, and isoniazid-induced MASs was highly effective in bioactivating coumarin producing LC50 and EC50 (malformation) values of 37.3 and 17.3 mg/L, respectively. Each of the MASs did not appreciably alter the TI values The β-naphthoflavone-induced MAS inhibited growth at 10.0 mg/L, whereas, the Aroclor 1254induced and mixed MASs inhibited growth at 25.0 mg/L. The isoniazid- and phenobarbitalinduced MASs tests did not appreciable alter growth compared to unactivated coumarin. Results from Test No. 2 were similar to results from Test No. 1 in which each of the Aroclor 1254induced, \( \beta\)-naphthoflavone-induced, and mixed MASs were successful in bioactivating coumarin, whereas, the phenobarbital- and isoniazid-induced systems were less effective and ineffective in bioactivating coumarin, respectively. Bioactivated coumarin induced microophthalmia. craniofacial abnormalities, visceral edema, and mouth defects. These abnormalities were similar to those recorded in previous studies and similar to those identified in rodents and rabbits, however somewhat more controversial than cyclophosphamide (40). As with cyclophosphamide, the results of the present study were not necessarily unexpected as coumarin has been shown to primarily bioactivated by aryl hydrocarbon hydroxylase isozymes (B-naphthoflavone-inducible P-450) (41).

Results of MAS performance evaluation studies with 2-AAF are provided in Table 4. Tests with unactivated 2-AAF produced 96-hour LC50 and EC50 (malformation), TI, and MCIG values of 125.9 mg/L, 8.7 mg/L, 14.5, and 125.0.0 mg/L. In Test No. 1, Aroclor 1254-induced MAS reduced the 96-hour LC50 and EC50 (malformation) values to 17.9 and 7.0 mg/L, respectively. Phenobarbital-induced MAS reduced the 96-hour LC50 and EC50 (malformation) values to a lesser extent to 75.4 and 12.1 mg/L, respectively. The \(\beta\)-naphthoflavone-induced MAS effectively bioactivated 2-AAF to 96-hour LC50 and EC50 (malformation) values of 17.4 and 7.2 mg/L, respectively. The isoniazid-induced MAS was also less effective in bioactivating 2-AAF producing LC50 and EC50 (malformation) values of 100.2 and 10.7 mg/L. The postisolation mixture of the phenobarbital-, \( \beta \)-naphthoflavone-, and isoniazid-induced MASs was highly effective in bioactivating 2-AAF producing LC50 and EC50 (malformation) values of 17.7 and 7.2 mg/L, respectively. Each of the MASs, with the exception of the isoniazid-induced MAS appreciably reduced the TI value of unactivated 2-AAF. The B-naphthoflavone-induced, Aroclor 1254-induced, and mixed MAS inhibited growth at 10.0 mg/L. The phenobarbital- and isoniazid-induced MAS tests inhibited growth at 50.0 and 100.0 mg/L, respectively. Results from Test No. 2 were similar to results from Test No. 1 in which each of the Aroclor 1254-

Table 3

Results of FETAX tests with Coumarin and Differently-Induced Exogenous

Metabolic Activation Systems (MASs)

Test No.	MAS	LC50 <sup>1</sup>	EC50 <sup>1, 2</sup>	TI <sup>3</sup>	MCIG <sup>4</sup>
					,
1	None	129.4	49.0	2.6	50.0
		(127.2-131.6)	(48.1-55.8)		
	Aroclor 1254	68.9	25.9	2.7	25.0
		(62.6-75.8)	(23.1-29.1)		
	Phenobarbital	93.3	51.5	1.8	50.0
		(90.7-95.9)	(43.1-61.6)		
	β-Naphthoflavone	57.2	16.9	3.4	10.0
		(51.8-63.2)	(15.0-19.6)		
	Isoniazid	98.0	46.1	2.1	50.0
		(94.6-101.5)	(43.2-51.2)		
	Mixed <sup>5</sup>	37.3	17.1	2.2	25.0
		(32.4-43.0)	(15.0-19.6)		

(continued)

Results of FETAX tests with Coumarin and Differently-Induced Exogenous **Metabolic Activation Systems (MASs)** 

(continued)

Test No.	MAS	LC50 <sup>1</sup>	EC50 <sup>1, 2</sup>	$\mathrm{TI}^3$	MCIG <sup>4</sup>
2	None	136.8 (130.6-143.2)	43.0 (38.0-47.1)	3.2	50.0
	Aroclor 1254	73.5 (68.4-79.0)	34.8 (31.0-39.1)	2.1	25.0
	Phenobarbital	94.8 (91.8-97.9)	53.2 (47.4-59.7)	1.8	50.0
	β- Naphthoflavone	64.0 (59.4-70.0)	19.7 (16.7-23.3)	3.3	10.0
	Isoniazid	106.6 (101.9-111.5)	48.4 (43.7-53.5)	2.2	50.0
	Mixed <sup>5</sup>	39.8 (35.1-45.1)	20.9 (18.5-23.4)	1.9	25.0

<sup>&</sup>lt;sup>1</sup> Expressed as mg/L.

<sup>2</sup> Malformation.

<sup>3</sup> Teratogenic Index calculated by dividing the 4-d LC50/4-d EC50 (malformation).

<sup>4</sup> Minimum Concentration to Inhibit Growth (Wiliams Test, P=0.05).

<sup>5</sup> Consists of equal post-isolation activity mix of phenobarbital-, β-naphthoflavone-, and isoniazid-induced rat liver microsomes.

Table 4

Results of FETAX tests with 2-Acetylaminofluorene and Differently-Induced Exogenous Metabolic Activation Systems (MASs)

Test No.	MAS	LC50 <sup>1</sup>	EC50 <sup>1,2</sup>	$\mathrm{TI}^3$	MCIG <sup>4</sup>
1	None	125.9 (120.8-131.8)	8.7 (6.4-8.0)	14.5	125.0
	Aroclor 1254	17.9 (17.4-18.4)	7.0 (6.1-8.0)	2.6	10.0
	Phenobarbital	75.4 (72.3-78.6)	12.1 (10.5-13.9)	6.2	50.0
	β-Naphthoflavone	17.4 (16.7-18.3)	7.2 (6.4-8.0)	2.4	10.0
	Isoniazid	100.2 (93.7-107.1)	10.7 (9.4-12.3)	9.4	100.0
	Mixed <sup>5</sup>	17.7 (15.9-19.7)	7.5 (6.5-8.6)	2.4	10.0

Table 4 (continued)

Results of FETAX tests with 2-Acetylaminofluorene and Differently-Induced

Exogenous Metabolic Activation Systems (MASs)

Test No.	MAS	LC50 <sup>1</sup>	EC50 <sup>1,2</sup>	ΤΓ³	MCIG <sup>4</sup>
2	None	130.7 (126.5-135.0)	9.1 (8.2-10.1)	14.4	125.0
	Aroclor 1254	22.0 (20.4-23.6)	6.4 (5.5-7.4)	3.4	10.0
	Phenobarbital	78.7 (73.5-84.2)	28.1 (15.0-21.5)	4.3	50.0
	β-Naphthoflavone	21.0 (19.3-22.9)	6.7 (5.9-7.7)	3.1	10.0
	Isoniazid	90.8 (84.5-97.6)	14.2 (12.3-16.4)	6.4	100.0
	Mixed <sup>5</sup>	18.0 (15.8-20.5)	7.1 (6.4-7.9)	2.5	10.0

<sup>1</sup> Expressed as mg/L.

<sup>2</sup> Malformation.

<sup>4</sup> Minimum Concentration to Inhibit Growth (William Test, P=0.05).

<sup>&</sup>lt;sup>3</sup> Teratogenic Index calculated by dividing the 4-d LC50/4-d EC50 (malformation).

<sup>&</sup>lt;sup>5</sup> Consists of equal post-isolation activity mix of phenobarbital-, β-naphthoflavone-, and isoniazid-induced rat liver microsomes.

induced, ß-naphthoflavone-induced, and mixed MASs were successful in bioactivating 2-AAF, whereas, the phenobarbital- and isoniazid-induced systems were less effective and ineffective in bioactivating 2-AAF, respectively. Bioactivated 2-AAF induced pericardial edema, craniofacial mal-development, skeletal kinking, and microencephaly. These abnormalities were similar to those recorded in previous FETAX studies and similar to those identified in rodents and rabbits (3). As with cyclophosphamide, the results of the present study were not necessarily unexpected as 2-AAF has been shown to primarily bioactivated by aryl hydrocarbon hydroxylase isozymes (ß-naphthoflavone-inducible P-450) (42).

Results of MAS performance evaluation with trichloroethylene is provided in Table 5. Unactivated trichloroethylene induced 96-hour LC50, EC50 (malformation), TI, and MCIG values of 412.0 mg/L, 40.9 mg/L, 10.1, and 152.0 mg/L. In Test No. 1, the Aroclor 1254induced MAS and the \( \beta\)-naphthoflavone-induced MASs each did not appreciably alter the 96hour LC50, EC50 (malformation), TI, and MCIG values. The phenobarbital-induced MAS only altered the 96-hour EC50 (malformation) by reducing it to 18.0 mg/L. The isoniazid-induced MAS reduced the 96-hour LC50 and EC50 (malformation) values to 190.4 and 11.9 mg/L, respectively. The TI value was increased to 16.0. The post-isolation mixture of the phenobarbital-, B-naphthoflavone-, and isoniazid-induced MASs was highly effective in bioactivating trichloroethylene producing LC50 and EC50 (malformation) values of 181.3 and 7.5 mg/L, respectively. The mixed MAS also produced the greatest TI value of 24.2 and inhibited growth at comparable levels (50.0 mg/L) to those observed with isoniazid-induced MAS tests. Results from Test No. 2 were similar to results from Test No. 1 in which each of the isoniazid-induced and mixed MASs were successful in bioactivating trichloroethylene, whereas, the \( \beta\)-naphthoflavone-, Aroclor 1254-, and to a lesser extent the phenobarbital-induced systems were less effective in bioactivating trichloroethylene. Bioactivated trichloroethylene induced mal-developed guts, microencephaly, hydroencepahaly, hypognathia, microophthalmia, abnormal mouth development, and muscular kinking. These abnormalities were similar to those recorded in previous studies and similar to those identified in rodents, rabbits, and monkeys (6). The results of the present study were not necessarily unexpected as trichloroethylene has been shown to primarily bioactivated by isoniazid-inducible P-450 (43).

Raw data from the FETAX testing is provided as Appendix A.

#### **Development of Metabolic Action System Test Kits**

In order to facilitate the use of a simplistic metabolic activation system to use outside of our laboratory which would benefit the U.S. Army, and the scientific community, a MAS test kit was developed and evaluated in the aforementioned studies. The MAS test kit was developed and refined during Phase I studies and is proposed for use in the interlaboratory validation study which encompasses Phase II work. A model of the MAS test kit is included as Appendix B.

Table 5

Results of FETAX tests with Trichloroethylene and Differently-Induced Exogenous Metabolic Activation Systems (MASs)

Test No.	MAS	LC50 <sup>1</sup>	EC50 <sup>1, 2</sup>	$\mathrm{TI}^3$	MCIG <sup>4</sup>
1	None	412.0 (370.4-458.3)	40.9 (35.4-42.3)	10.1	152.0
	Aroclor 1254	378.1 (339.5-421.4)	40.2 (34.6-46.6)	9.4	152.0
	Phenobarbital	412.1 (347.67-437.75)	18.0 (14.6-22.3)	22.9	152.0
	β-Naphthoflavone	390.1 (349.7-437.8)	39.7 (34.0-46.3)	9.8	152.0
	Isoniazid	190.4 (167.3-216.6)	11.9 (8.5-16.7)	16.0	50.0
	Mixed <sup>5</sup>	181.3 (158.8-206.9)	7.5 (6.5-8.7)	24.2	50.0

(continued)

Table 5 (continued) Results of FETAX tests with Trichloroethylene and Differently-Induced Exogenous Metabolic Activation Systems (MASs)

st No	MAS	LC50	EC501,2	$-\mathbf{TI}^3$	MCIG
2	None	426.0 (384.5-472.9)	36.2 (30.8-42.6)	11.8	152.0
	Aroclor 1254	383.5 (341.6-430.5)	37.3 (32.0-43.4)	10.3	152.0
	Phenobarbital	410.8 (367.17 <b>-</b> 459.6)	19.5 (16.4-23.1)	21.1	97.0
	β-Naphthoflavone	388.9 (344.6-438.9)	40.2 (34.2-47.3)	9.7	152.0
	Isoniazid	175.3 (153.4-200.2)	11.5 (8.7-15.2)	15.2	50.0
	Mixed <sup>5</sup>	169.1 (147.8-193.5)	7.6 (6.4-9.1)	22.3	50.0

Minimum Concentration to Inhibit Growth (Williams Test, P=0.05).

<sup>&</sup>lt;sup>1</sup> Expressed as mg/L.
<sup>2</sup> Malformation.

<sup>&</sup>lt;sup>3</sup> Teratogenic Index calculated by dividing the 4-d LC50/4-d EC50 (malformation).

<sup>&</sup>lt;sup>5</sup> Consists of equal post-isolation activity mix of phenobarbital-, β-naphthoflavone-, and isoniazid-induced rat liver microsomes.

# **Conclusions**

Results from these studies suggested that the Aroclor 1254-induced MAS could effectively be replaced by a mixed lot of phenobarbital-, \(\beta\)-naphthoflavone-, and isoniazid-induced rat liver microsomes. In addition, the use of a MAS test kit is a practicable means of facilitating external use of the system outside of our laboratory, particularly by the U.S. Army. Overall, the results from these studies suggested that continued validation of an exogenous metabolic activation system for FETAX is warranted in Phase II SBIR Studies to increase the overall predictability of the assay, and thus acceptance by the scientific and regulatory communities.

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# **Bibliography**

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- 1. D.J. Fort, T.L. Propst, B.C. Faulkner, E.L. Stover, J.A. Bantle, and R.A. Finch. Utilization of a broad-spectrum of induced rat liver microsomes is an effective metabolic activation system for FETAX, Drug Chem. Toxicol., in preparation.
- 2. D.J. Fort, T.L. Propst, B.C. Faulkner, E.L. Stover, J.A. Bantle, and R.A. Finch. Utilizing differently induced and inhibited rat liver microsomes to evaluate modes of biotransformation and detoxification, J. Appl. Toxicol., in preparation.

#### **Abstracts and Presentations**

- 1. D.J. Fort. Applications of FETAX, NIEHS FETAX Workshop, RTP, NC, April 1997.
- 2. D.J. Fort, T. Propst, amd E.L. Stover. Evaluating Mechanisms of Action of Development Toxicants Using FETAX as a Model, Society of Toxicology, Cincinnati, OH, March 1997.
- 3. D.J. Fort, T. Propst, and E.L.Stover. Evaluating Biotransformation of Development Toxicants Using FETAX as a Model, Teratology Society, Palm Beach, FL, June 1997.
- 4. D.J. Fort, E.L. Stover, T. Propst, B. Faulkner, J.A. Bantle, and R. Finch. Validation of a Whole-Embryo Limb Development Assay Using *Xenopus laevis*. Society of Environment Toxicology and Chemistry (SETAC), San Francisco, CA, November 1997.

## **Project Personnel**

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Douglas J. Fort, Ph.D.

Kendall W. King, P.E.

John Scott

Susan Schaefer

Pat Lee

**Timothy Propst** 

Brian Faulkner

Curtis Mirkes

Scott Shannon

Tommy Wynn

John Delphon

Micheal Copenhaver

Appendix A

Raw Data

F	ETAX SU	MMARY	SHEET		Test N	io. 1	_					
Test Material	imar,	$\cap$		Inv	estigator T	ont-						
Source US A		SBIE	>	Lab	5 <del>1</del>							
CAS No.		Lot No.		Tes	Start Date							
Composition/Purity				Test	End Date		-					
Solvent		Conc.		Test	: Units (i.e., m	g/ml) m	g/. L					
	DAY 1	DA	Y 2	DAY 3	DAY 4		DAY 5					
— pH — Stock												
Control												
Highest Conc.												
FETAX CONTROL No. Dead or Malformed	X 100 = 9	<b>%</b>	= MORTAI RECO		MAI	FORMA' RECORD	i i					
Total Number		il	: <u>80</u> x 10	0 =9	6 <u>0 : R</u>	<u>0</u> : <u>60</u> x 100 = <u>0</u> %						
Solvent Control		:	X 10	0 =9	s:	X 100	X 100 =%					
Control Length	nm	Solve	Solvent Control Length mm									
Minimum Concentration t	o Inhibit Gr	owth (MCI	G)									
UNNTINACO FEST MATERIAL/C	BNF/11	NIH M I	X/Arov SULTS	r/PB								
TEST MORTA	LITY	MALFO	RMATION	ST	STATISTICAL TEST USED							
NOEL												
LOEL	`	93.32				.5	1.49					
LC50 129.36/57.19/	78.0/37.3	33/68.87	EC50 4	9.01/16	1/16.44/46.13/47.14/25.89/							
95% Confidence limits 9. 127.19 - 131.56/51.	1.64-101.49/3 15-63.20	32.43-42.97 62.61=5.15	95% Con	fidence Li 70/14.9	mits 48,08 - 5 1 - 19.162/	5.77/14.8 23.07-29	9-19,27/ 9.05/					
TEST TERATO							43.06-					
90.72-95	:92						•					
OSITIVE CONTRO	L: 6 AMIN	NONICO.	TINAMII	DE (6-A	N) RESUL	.TS						
CONCENTRATION		MORTA	LITY		MALFO	DRMATIC	N					

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CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	:X 100 =%	:X 100 =%
2500 mg/L	:X 100 =%	:X 100 =%

# FETAX MORTALITY DATA

II.

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THE PAIN OF THE PA	Test Material (STATALL)	FETAX (ml) 24 hr 48 hr 72 hr 96 hr Total No %		-			0 0 0	0 0 0	0 0 0	0 0 0 0	0 0 0	ρ ο ο ο	2 2 1	20,20 40	1 20 20 40	D	000	50	5 6 //	20 20 40	0 0 0	0 0 0	0 0	0 0 0	325	9 9 0	1
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Investigator	Date	Concentration	X	文	MAS	Ţ	27	Under.		0 - 0	0 =	\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\		000	1	25	2 .	1	2 >		2	777	7 7	8 6	9	9	0

FETAX MORTALITY DATA

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				Test Number	umber					
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27				-			2	ז		
- 4							5:1	7/		
2 >							11:17	7.8		
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FETAX MALFORMATION DATA

Investigator		
Date	Test Material	
	Test Number	
	NOLLE ATTENDED	
MALFORMATION	170	
Severe		TOTAL
Stunted		
Gut		
Edema		
Multiple		
Cardlac		
Abdominal		
Facial		
Cephalle		
Optic		
Tall		
Notochord		
Fin		
Face		
Eye		
Brain		
Hemorrhage		
Cardiac		
Bilsters		
Other-specify		
No. Malformed		
Total No.	10 40 - 10 27 40 100 25 4 10 10 8 43 35 24 - 0 13	
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FETAX MALFORMATION DATA

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Investigator		Date			MALFORMATION	Severe	Stunted	Gut	Edema	Multiple	Cardiac	Abdominat	Facial	Cephalle	Optic	Tall	Notochord	Fin	Face	Eye	Brain	Hemorrhage	Cardiac	Bilsters	Other-specify	No. Malformed	Total No.	Commen(s:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 1/30/97 TEST NUMBER: 1 DURATION: 4 D

CHEMICAL: COUMARIN UNACT.

SPECIES: FETAX

RAW DATA:

SPEARMAN-KARBER ESTIMATES: LC50: 129.36

VAR OF LN OF EST. : .71284D-04

95% LOWER CONFIDENCE: 127.19 95% UPPER CONFIDENCE: 131.56

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 1/30/97 TEST NUMBER: 1 DURATION: 4 D CHEMICAL: COUMARIN SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 20.00 27.00 60.00 86.00 .00 .00 .00 NUMBER EXPOSED: 40 40 40 40 0 0 0 0 0 0 0 MORTALITIES: 0 5 11 40 0 0 0 0 0

SPEARMAN-KARBER TRIM:

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SPEARMAN-KARBER ESTIMATES: LC50: 57.19

VAR OF LN OF EST. : .24975D-02

95% LOWER CONFIDENCE: 51.75 95% UPPER CONFIDENCE: 63.20

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TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 1/30/97 TEST NUMBER: 1 DURATION: 4 D

CHEMICAL: COUMARIN INH

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 60.00 86.00 100.00 115.00 .00 .00 .00

NUMBER EXPOSED: 40 40 40 40 0 0 0 0

MORTALITIES: 0 5 16 40 0 0 0 0 0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 98.00

VAR OF LN OF EST. : .30504D-03

95% LOWER CONFIDENCE: 94.64 95% UPPER CONFIDENCE: 101.49

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 1/30/97 TEST NUMBER: 1 DURATION: 4 D CHEMICAL: COUMARIN SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 5.00 20.00 27.00 60.00 86.00 .00 .00 NUMBER EXPOSED: 40 40 40 40 40 0 0 0 0 MORTALITIES: 0 4 12 28 40 0 0 0 0 SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 37.33

VAR OF LN OF EST. : .49454D-02

95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 1/30/97 TEST NUMBER: 1 DURATION: 4 D

CHEMICAL: COUMARIN AROCLOR

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 20.00 27.00 60.00 86.00 100.00 .00 .00

NUMBER EXPOSED: 40 40 40 40 0 0 0

MORTALITIES: 0 3 8 22 40 0 0 0 0

.00% SPEARMAN-KARBER TRIM:

SPEARMAN-KARBER ESTIMATES: LC50: 68.87

VAR OF LN OF EST. : .22691D-02

95% LOWER CONFIDENCE:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719;

CORRECTION 12(4):417 (1978).

DATE: 1/30/97 TEST NUMBER: 1 DURATION: 4 D

CHEMICAL: COUMARIN PB

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 60.00 86.00 100.00 115.00 100.00 .00 .00 .

NUMBER EXPOSED: 40 40 40 40 0 0 0 0

MORTALITIES: 0 3 33 40 40 0 0 0 0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 93.32

VAR OF LN OF EST. : .18934D-03

95% LOWER CONFIDENCE: 90.79 95% UPPER CONFIDENCE: 95.92

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719;

DATE: 1/31/97 TEST NUMBER: 1

CORRECTION 12(4):417 (1978).

CHEMICAL: COUMARIN

SPECIES: FETAX

DURATION: 4 D

RAW DATA:

CONCENTRATION(MG/L ) 5.00 20.00 27.00 60.00 86.00 .00 .00 NUMBER EXPOSED: 40 40 40 40 0 0 0 0 MORTALITIES: 0 2 10 14 40 0 0 0 0 SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 49.01

VAR OF LN OF EST. : .41671D-02

95% LOWER CONFIDENCE: 43.08 95% UPPER CONFIDENCE: 55.77

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 1/31/97 TEST NUMBER: 1 DURATION: 4 D

CHEMICAL: COUMARIN BNF

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 5.00 20.00 27.00 60.00 86.00 .00 .00 NUMBER EXPOSED: 40 40 35 29 40 0 0 0 0 MORTALITIES: 0 15 35 29 40 0 0 0 0

SPEARMAN-KARBER TRIM:

.00%

SPEARMAN-KARBER ESTIMATES: EC50:

VAR OF LN OF EST. : .41659D-02

95% LOWER CONFIDENCE: 95% LOWER CONFIDENCE: 14.89 95% UPPER CONFIDENCE: 19.27

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719;

CORRECTION 12(4):417 (1978).

DATE: 1/31/97 TEST NUMBER: 1 DURATION: 4 D

CHEMICAL: COUMARIN INH

SPECIES: FETAX

RAW DATA:

CONCENTRATION(4 ) 20.00 27.00 60.00 86.00 86.00 .00 .00 NUMBER EXPOSED: 40 40 40 35 40 0 0 0 0 MORTALITIES: 0 8 23 35 40 0 0 0 0 CERTARMAN-KARRED TRIM.

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 46.13

VAR OF LN OF EST. : .32569D-02

95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE: 51.70

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719;

CORRECTION 12(4):417 (1978).

DATE: 1/31/97 TEST NUMBER: 1 DURATION: 4 D CHEMICAL: COUMARIN MIX

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 5.00 20.00 27.00 60.00 86.00 .00 .00 NUMBER EXPOSED: 40 36 28 12 40 0 0 0 0 MORTALITIES: 0 3 16 12 40 0 0 0 0 SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 27.41

VAR OF LN OF EST. : .41478D-02

95% LOWER CONFIDENCE: 24.10 95% UPPER CONFIDENCE:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 1/31/96 TEST NUMBER: 1 DURATION: 4 D CHEMICAL: COUMARIN SPECIES: FETAX MIX

RAW DATA:

CONCENTRATION(MG/L ) 5.00 20.00 27.00 60.00 86.00 .00 .00 NUMBER EXPOSED: 40 36 28 32 18 0 0 0 0 MORTALITIES: 0 13 28 24 18 0 0 0 0 0 SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50:

VAR OF LN OF EST. : .45564D-02

95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE: 19.62

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719;

CORRECTION 12(4):417 (1978).

DATE: 1/31/97 TEST NUMBER: 1 DURATION: 4 D CHEMICAL: COUMARIN

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 5.00 20.00 27.00 60.00 86.00 .00 .00

NUMBER EXPOSED: 40 40 40 32 18 0 0 0

MORTALITIES: 0 4 26 32 18 0 0 0 0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 25.89

VAR OF LN OF EST. : .33158D-02

95% LOWER CONFIDENCE: 23.07 95% UPPER CONFIDENCE: 29.05

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 1/31/97 TEST NUMBER: 1 DURATION: 4 D

CHEMICAL: COUMARIN PB

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 27.00 60.00 86.00 100.00 86.00 .00 .00

NUMBER EXPOSED: 40 40 37 7 18 0 0 0

MORTALITIES: 8 22 29 7 18 0 0 0 0

SPEARMAN-KARBER TRIM: 20.00%

SPEARMAN-KARBER ESTIMATES: EC50: 51.49

VAR OF LN OF EST. : .80018D-02

43.06 95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE:

## FETAX MORTALITY DATA

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# FETAX MORTALITY DATA

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FETAX MALFORMATION DATA

Test Material   Test Materia			TOTAL									
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2 20 27 60 84 100 115 150  2 20 27 60 84 100 140 23 11 11 11 11 11 11 11 11 11 11 11 11 11	Test Material	Test Number	77									13 30 33 - 3
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FETAX MALFORMATION DATA

Investigator	30 - 31 - 31 - 31 - 31 - 31 - 31 - 31 -	
	Test Material	
	Test Number	
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MALFURNATION	20 27 60 5 20 27 60 86 5 30 27 12 06	
Severe		TOTAL
Stunted		
Gut		
Edema		
Multiple		
Cardiac		
Abdominal		
Facial		
Cephalle		
Optic		
Tall		
Notochord		
Fin		
Face		
Eye		
Brain		
Hemorrhage		
Cardiac		
Blisters		
Other-specify		
rmed	0 1 25 13 0 1 15 20 18 0 0 0 0 19 13	
Total No.	3 27 13 40 62 62 62 62 17 62 17 8	
	0 C   22   1	

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 1/31/97 TEST NUMBER: 1 DURATION: 4 D

CHEMICAL: COUMARIN PB

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 20.00 27.00 60.00 86.00 100.00 .00 .00 .

NUMBER EXPOSED: 40 40 40 36 13 0 0 0

MORTALITIES: 0 6 19 28 13 0 0 0 0

SPEARMAN-KARBER TRIM: .00%

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SPEARMAN-KARBER ESTIMATES: EC50: 53.17

VAR OF LN OF EST. : .33669D-02

95% LOWER CONFIDENCE: 47.35 95% UPPER CONFIDENCE: 59.72

FOR REFERENCE, CITE: HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 1/31/97 TEST NUMBER: 2 DURATION: 4 D

CHEMICAL: COUMARIN MIX

SPECIES: FETAX

RAW DATA:

SPEARMAN-KARBER ESTIMATES: EC50: 20.80

VAR OF LN OF EST. : .34513D-02

95% LOWER CONFIDENCE: 18.50 95% UPPER CONFIDENCE: 23.40

FOR REFERENCE, CITE: HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 1/31/96 TEST NUMBER: 2 DURATION: 4 D

CHEMICAL: COUMARIN AROCLOR

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 5.00 20.00 27.00 60.00 86.00 .00 .00 .

NUMBER EXPOSED: 40 40 40 35 15 0 0 0

MORTALITIES: 0 1 15 30 15 0 0 0 0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 34.84

VAR OF LN OF EST. : .33751D-02

95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE: 31.02 39.13

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 1/31/97 TEST NUMBER: 2 DURATION: 4 D

CHEMICAL: COUMARIN UNACT.

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 5.00 20.00 27.00 60.00 86.00 .00 .00 NUMBER EXPOSED: 40 40 40 40 40 0 0 0 0 MORTALITIES: 0 0 8 29 40 0 0 0 SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 42.29

VAR OF LN OF EST. : .28794D-02

95% LOWER CONFIDENCE: 37.99 95% UPPER CONFIDENCE: 47.08

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 1/31/97 TEST NUMBER: 2 DURATION: 4 D

CHEMICAL: COUMARIN BNF

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 5.00 20.00 27.00 60.00 86.00 .00 .00 NUMBER EXPOSED: 40 40 39 33 40 0 0 0 0 MORTALITIES: 2 13 30 33 40 0 0 0

SPEARMAN-KARBER TRIM: 5.00%

SPEARMAN-KARBER ESTIMATES: EC50:

VAR OF LN OF EST. : .71002D-02

ST.: .71002D-02 95% LOWER CONFIDENCE:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 1/31/97 TEST NUMBER: 2 DURATION: 4 D

CHEMICAL: COUMARIN INH

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 20.00 27.00 60.00 86.00 100.00 .00 .00 NUMBER EXPOSED: 40 40 40 35 29 0 0 0 MORTALITIES: 0 3 25 34 29 0 0 0 SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 48.35

VAR OF LN OF EST. : .25411D-02

95% LOWER CONFIDENCE: 43.71 95% UPPER CONFIDENCE: 53.48

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 1/31/97 TEST NUMBER: 2 DURATION: 4 D

CHEMICAL: COUMARIN SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 100.00 115.00 150.00 200.00 86.00 .00 .00 NUMBER EXPOSED: 40 40 40 18 0 0 0 0 MORTALITIES: 0 7 29 40 18 0 0 0 0 SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 136.78

VAR OF LN OF EST. : .52994D-03

95% LOWER CONFIDENCE: 130.63 95% UPPER CONFIDENCE: 143.23

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 1/31/97 TEST NUMBER: 2 DURATION: 4 D CHEMICAL: COUMARIN SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 20.00 27.00 60.00 86.00 86.00 .00 .00 NUMBER EXPOSED: 40 40 40 18 0 0 0 MORTALITIES: 0 1 7 40 18 0 0 0

SPEARMAN-KARBER TRIM:

.00%

SPEARMAN-KARBER ESTIMATES: LC50:

VAR OF LN OF EST. : .13949D-02

95% LOWER CONFIDENCE:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 1/31/97 TEST NUMBER: 2 DURATION: 4 D CHEMICAL: FETAX SPECIES: COUMARIN

RAW DATA:

CONCENTRATION(MG/L ) 60.00 86.00 100.00 115.00 150.00 .00 .00 NUMBER EXPOSED: 40 40 40 40 40 0 0 0 0 MORTALITIES: 0 5 11 27 40 0 0 0 SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 106.60

VAR OF LN OF EST. : .50901D-03

95% LOWER CONFIDENCE: 101.89 95% UPPER CONFIDENCE: 111.52

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 1/31/97 TEST NUMBER: 1 DURATION: 4 D CHEMICAL: COUMARIN SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 5.00 20.00 27.00 60.00 86.00 .00 .00 NUMBER EXPOSED: 40 40 40 40 0 0 0 0 MORTALITIES: 0 1 13 27 40 0 0 0 0 SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 39.80

VAR OF LN OF EST. : .39283D-02

95% LOWER CONFIDENCE: 35.11 95% UPPER CONFIDENCE: 45.11

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 1/31/97 TEST NUMBER: 2 DURATION: 4 D CHEMICAL: FETAX SPECIES: COUMARIN

RAW DATA:

CONCENTRATION(MG/L ) 27.00 60.00 86.00 100.00 86.00 .00 .00 NUMBER EXPOSED: 40 40 40 40 40 0 0 0 0 MORTALITIES: 0 5 25 40 40 0 0 0 0 SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 73.53

VAR OF LN OF EST. : .12997D-02

95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE: 68.42 79.03

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719;

CORRECTION 12(4):417 (1978).

DATE: 1/31/97 TEST NUMBER: 2 DURATION: 4 D

CHEMICAL: COUMARIN

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 60.00 86.00 100.00 115.00 86.00 .00 .00 NUMBER EXPOSED: 40 40 40 40 0 0 0 0 MORTALITIES: 0 4 27 40 40 0 0 0 0 SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 94.77

VAR OF LN OF EST. : .26255D-03

95% LOWER CONFIDENCE: 95% LOWER CONFIDENCE:91.7595% UPPER CONFIDENCE:97.89

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FETAX MORTALITY DATA

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			FETAX (ml)																														
-		28/97	Stock (mf)																														
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FETAX MORTALITY DATA

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Test Maria		lest Number	1)   24 hr   49 hr																						-
			Stock (ml)																						
Investigator	Date		Concentration	Areda O. h		70	200	N K	70		200	र प	207	2		KNAC DANA	2	2,	35	50	57	0	77	0	7

FETAX MALFORMATION DATA

Investigator	A LAG MOLECULAR DE LA MARIA DEL LA MARIA DEL LA MARIA DEL LA MARIA DEL LA MARIA DEL LA MARIA DELLA DEL	
	Test Material	ハームハイ
DAIC	Test Number	
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		TOTAL
Stunted		
Gut		
Edema		
Multiple		
Cardlac		
Abdominal		
Facial		
Cephalic		
Optic		
Tall		
Notochord		
Fln		
Face		
Eye		
Brain		
Hemorrhage		
Cardiac		
Bilsters		
Other-specify		
rmed	100	
	4038 33 6 3 46	
Comments:		

FETAX MALFORMATION DATA

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Abdominal				-	+	+	+	1	+	$\perp$	_		1	$\dagger$	+	+	+	+	_	$\downarrow$	1			
Facial		1	T	<del> </del>	$\dagger$	$\dagger$	<del> </del>	1	_	+			İ	$\dagger$	+	+	+	+	_		1			
Cephalic		Π		İ	<del> </del>	+-	+		+	_	1		İ	+	+	$\frac{\perp}{1}$	+	_	1	$\downarrow$	_			
Optic					$\vdash$	$\vdash$	+	+	+-	-	1			+	+	+	+	+	+	_				
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Fin					$\dagger$	-	+	+	+	+	1			$\dagger$	+	+	+	+	$\downarrow$	4				
Fасе					+	+	+	+	+	+	1			$\dagger$	+	+	+	+	+		1			
Eye					+	+	+	+	<u> </u>	+			İ	$\dagger$	+	$\dashv$	-	+	4	$\downarrow$	1			
Brain				T	-	+	+	+	+-	+	1			$\dagger$	+	+	+	+		$\perp$	$\perp$			
Hemorrhage		T	T		$\dagger$	+-	+	+	+	+				$\dagger$	+	+		+	$\perp$	1	$\perp$			
Cardiac					-	$\frac{1}{1}$	+	+	+	-	1			$\dagger$	╁	+	-	+	+	_	1			
Blisters					<del> </del>	+	-	+	+	_			İ	+	+	+	+	$\perp$	+	_	1			
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Commentr											1			2	H	9	2 79	4	3027	17				

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 2/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: FETAX
SPECIES: 2-AAF

RAW DATA:

SPEARMAN-KARBER ESTIMATES: LC50: 125.90

VAR OF LN OF EST. : .52637D-03

95% LOWER CONFIDENCE:120.2695% UPPER CONFIDENCE:131.81

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 2/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: 2-AAF PB
SPECIES: FETAX

RAW DATA:

SPEARMAN-KARBER ESTIMATES: LC50: 75.38

VAR OF LN OF EST. : .42497D-03

95% LOWER CONFIDENCE:72.3395% UPPER CONFIDENCE:78.55

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 2/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: 2-AAF
SPECIES: FETAX

RAW DATA:

SPEARMAN-KARBER TRIM:

.00왕

SPEARMAN-KARBER ESTIMATES: LC50: 17.41

VAR OF LN OF EST. : .56294D-03

95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE: 18.25

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 2/3/97 TEST NUMBER: 1 DURATION: 4 D

CHEMICAL: 2-AAF INH

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 50.00 57.00 110.00 125.00 110.00 125.00 .00 NUMBER EXPOSED: 40 40 40 40 40 40 0 MORTALITIES: 0 3 13 40 39 40 0 0 SPEARMAN-KARBER TRIM: .00% 0

SPEARMAN-KARBER ESTIMATES: LC50: 100.21

VAR OF LN OF EST. : .11150D-02

93.73 107.13 95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 2/3/97 TEST NUMBER: 1 DURATION: 4 D CHEMICAL: 2-AAF AROCLOR

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 9.60 11.00 30.00 125.00 110.00 125.00 .00 NUMBER EXPOSED: 40 40 40 40 40 40 0 0 0 MORTALITIES: 0 1 40 40 39 40 0 0 0 SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 17.91

VAR OF LN OF EST. : .19779D-03

17.41 95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE: 18.42

FOR REFERENCE, CITE: HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 2/3/97 TEST NUMBER: 1 DURATION: 4 D

CHEMICAL: 2-AAF MIX

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 3.00 9.60 11.00 30.00 35.00 50.00 .00 .

NUMBER EXPOSED: 40 40 40 40 40 0 0 0

MORTALITIES: 0 2 7 34 37 40 C 0 0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 17.70

VAR OF LN OF EST. : .28534D-02

15.90 19.69 95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE: .\_\_\_\_\_

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 2/3/97 TEST NUMBER: 1 DURATION: 4 D

CHEMICAL: 2-AAF UNACT.

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 3.00 9.60 11.00 30.00 35.00 50.00 .00 NUMBER EXPOSED: 40 40 40 40 40 40 0 0 0 MORTALITIES: 1 11 19 39 40 40 0 0 0 SPEARMAN-KARBER TRIM: 2.50%

SPEARMAN-KARBER ESTIMATES: EC50: 8.66

VAR OF LN OF EST. : .73618D-02

95% LOWER CONFIDENCE:
95% UPPER CONFIDENCE:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 2/3/97 TEST NUMBER: 1 DURATION: 4 D CHEMICAL: 2-AAF PB SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 3.00 9.60 11.00 30.00 35.00 50.00 .00 NUMBER EXPOSED: 40 40 40 40 40 0 0 0 MORTALITIES: 3 5 23 40 40 40 0 0 0 SPEARMAN-KARBER TRIM:

7.50%

SPEARMAN-KARBER ESTIMATES: EC50:

VAR OF LN OF EST. : .50053D-02

95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 2/3/97 TEST NUMBER: 1 DURATION: 4 D CHEMICAL: 2-AAF SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 3.00 9.60 11.00 30.00 35.00 50.00 .00 NUMBER EXPOSED: 40 40 37 40 40 40 0 0 0 MORTALITIES: 1 22 37 40 40 40 0 0 0 0

2.50% SPEARMAN-KARBER TRIM:

SPEARMAN-KARBER ESTIMATES: EC50: 7.16

VAR OF LN OF EST. : .31200D-02

6.41 95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE: 8.01

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 2/3/97 TEST NUMBER: 1 DURATION: 4 D CHEMICAL: 2-AAF INH

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 3.00 9.60 11.00 30.00 35.00 50.00 .00 NUMBER EXPOSED: 40 40 40 40 40 40 0 0 0 MORTALITIES: 1 10 25 40 40 40 0 0 0

SPEARMAN-KARBER TRIM: 2.50%

SPEARMAN-KARBER ESTIMATES: EC50: 10.74

VAR OF LN OF EST. : .45112D-02

95% LOWER CONFIDENCE: 9.39 95% UPPER CONFIDENCE: 12.28

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 2/3/97 TEST NUMBER: 1 DURATION: 4 D

CHEMICAL: 2-AAF AROCLOR

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 3.00 9.60 11.00 30.00 35.00 50.00 .00 NUMBER EXPOSED: 40 40 40 40 40 40 0 0 0 MORTALITIES: 3 23 40 40 40 40 0 0 0

SPEARMAN-KARBER TRIM: 7.50%

SPEARMAN-KARBER ESTIMATES: EC50: 6.98

VAR OF LN OF EST. : .43573D-02

95% LOWER CONFIDENCE: 6.11 95% UPPER CONFIDENCE: 7.96

FOR REFERENCE, CITE: HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 2/3/97 TEST NUMBER: 1 DURATION: 4 D

CHEMICAL: 2-AAF MIX

SPECIES: FETAX

RAW DATA:

SPEARMAN-KARBER TRIM: 5.00%

SPEARMAN-KARBER ESTIMATES: EC50: 7.48

VAR OF LN OF EST. : .51617D-02

95% LOWER CONFIDENCE: 6.48 95% UPPER CONFIDENCE: 8.64

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<u> </u>	Investigator	Fort			Test N	Test Material	2-AAF	生				
<u>'</u>	Date				Test N	Test Number	7					
	Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total	-	ş °X		
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FETAX MORTALITY DATA

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Test Number   2   1   1   1   1   1   1   1   1   1		Investigator				Test M	Test Material	7	-			
Concentration  Start (mt)  Att + MAS - 1.N.   48 hr   72 hr   48 hr   72 hr   48 hr   72 hr   48 hr   72 hr   48 hr   72 hr		Date				Test	l	7-2	ME			
MAS -   N   MAS -   MAS -						1 1631	umoer		7			
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FETAX MORTALITY DATA

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FETAX MALFORMATION DATA

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FETAX MALFORMATION DATA

Investigator		
Date	Test Material 3 - A.C.	
	Test Number	-
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Severe	11 30 3550 57 110 125 M CP-3 9.6 11 30 M CP AM-3 9.6 11 30 37 TOTAL	TAL
Stunted		
Gut		
Edema		
Multiple		
Cardiac		
Abdominal		
Facial		
Cephalle		
Optic		
Tall		
Notochord		
Fln		
Face		
Eye		
Brain		
Hemorrhage		
Cardiac		
Bilsters		
Other-specify		
No. Malformed		
Total No.	40 40 40 28 23 30 40 th 20 21 0 10 40 0 20 32 12	
Comments:	(	
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# FETAX MALFORMATION DATA

Investigator	AIAG NOI Chino	
Date	Test Material 2-4AF	
	Test Number	1
	UNATA. CONCENTRATION	
MALFORMATION	50 (7) 100 100 100 100 100 100 100 100 100 10	
Severe		AL.
Stunted		
Gut		
Edema		
Multiple		
Cardiac		
Abdominal		
Facial		
Cephalle		
Optic		
Tall		
Notochord		
Fla		
Face		
Eye		
Brain		
Hemorrhage		
Cardlac		
Bilisters		
Other-specify		
No. Malformed	10,	
Total No.		
Comments		
		1

# FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE:

TEST NUMBER: PB-MAS
DURATION: 4 D
CHEMICAL: 2-AAF
SPECIES: XENOPUS

### RAW DATA:

CONCENTRATION(MG/L ) 3.00 9.60 11.00 30.00 35.00 50.00 .00 .

NUMBER EXPOSED: 40 40 40 40 40 0 0 0

MORTALITIES: 0 1 6 23 40 40 0 0 0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 28.08

VAR OF LN OF EST. : .33379D-02

95% LOWER CONFIDENCE: 25.02 95% UPPER CONFIDENCE: 31.52

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

# FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

### DATE:

TEST NUMBER: BNF-MAS

DURATION: 4 D CHEMICAL: 2-AAF SPECIES: XENOPUS

### RAW DATA:

CONCENTRATION(MG/L ) 3.00 9.60 11.00 30.00 35.00 50.00 .00 NUMBER EXPOSED: 40 40 39 40 40 40 0 0 0 MORTALITIES: 3 25 39 23 40 40 0 0 0

SPEARMAN-KARBER TRIM:

7.50%

SPEARMAN-KARBER ESTIMATES: EC50: 6.72

VAR OF LN OF EST. : .42059D-02

95% LOWER CONFIDENCE: 5.90 95% UPPER CONFIDENCE: 7.65

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE:

TEST NUMBER: INH-MAS

DURATION: 4 D CHEMICAL: 2-AAF SPECIES: XENOPUS

RAW DATA:

CONCENTRATION(MG/L ) 3.00 9.60 11.00 30.00 35.00 50.00 .00 NUMBER EXPOSED: 40 40 40 40 40 0 0 0 MORTALITIES: 0 11 36 40 40 40 0 0 0 SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 9.10

VAR OF LN OF EST. : .28339D-02

95% LOWER CONFIDENCE: 8.18 95% UPPER CONFIDENCE: 10.12

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE:

TEST NUMBER: ARO-MAS

DURATION: 4 D

CHEMICAL: 2-AAF SPECIES: XENOPUS

RAW DATA:

CONCENTRATION(MG/L ) 3.00 9.60 11.00 30.00 35.00 50.00 .00 .

NUMBER EXPOSED: 40 39 31 40 40 40 0 0 0

MORTALITIES: 5 26 31 40 40 40 0 0 0

SPEARMAN-KARBER TRIM: 12.50%

6.38 ARO SPEARMAN-KARBER ESTIMATES: EC50: VAR OF LN OF EST. : .57942D-02

95% LOWER CONFIDENCE: 5.48

95% UPPER CONFIDENCE: 7.43

FOR REFERENCE, CITE: HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE:

TEST NUMBER: PB/INH/BNF-MAS

DURATION: 4 D CHEMICAL: 2-AAF SPECIES: XENOPUS

RAW DATA:

CONCENTRATION(MG/L ) 3.00 9.60 11.00 30.00 35.00 50.00 .00 NUMBER EXPOSED: 40 35 32 40 40 40 0 0 0 MORTALITIES: 0 20 32 40 40 40 0 0 .00%

SPEARMAN-KARBER TRIM:

SPEARMAN-KARBER ESTIMATES: EC50: 7.09

VAR OF LN OF EST. : .29530D-02

95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE: 7.90

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE: HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE:

TEST NUMBER: UNACT DURATION: 4 D CHEMICAL: 2-AAF SPECIES: XENOPUS

RAW DATA:

CONCENTRATION (MG/L ) 3.00 9.60 11.00 30.00 35.00 50.00 .00 NUMBER EXPOSED: 40 40 40 40 40 0 0 0 MORTALITIES: 1 5 20 32 40 40 0 0 MORTALITIES:

SPEARMAN-KARBER TRIM: 2.50%

SPEARMAN-KARBER ESTIMATES: EC50: 14.17 WAGT VAR OF LN OF EST. : .52331D-02

95% LOWER CONFIDENCE: 12.26 95% UPPER CONFIDENCE: 16.38

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE:

TEST NUMBER: PB-MAS DURATION: 4 D CHEMICAL: 2-AAF SPECIES: XENOPUS

RAW DATA:

CONCENTRATION(MG/L ) 35.00 50.00 57.00 110.00 125.00 .00 .00 .

NUMBER EXPOSED: 40 40 40 40 0 0 0 0

MORTALITIES: 0 1 7 33 40 0 0 0 0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 78.68

VAR OF LN OF EST. : .11536D-02

95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE: 73.51

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.

ENVIRON. SCI. TECHNOL. 11(7): 714-719;

CORRECTION 12(4):417 (1978).

DATE:

TEST NUMBER: BNF-MAS

DURATION: 4 D CHEMICAL: 2-AAF SPECIES: XENOPUS

RAW DATA:

CONCENTRATION(MG/L ) 9.60 11.00 30.00 35.00 125.00 .00 .00 NUMBER EXPOSED: 40 40 40 40 40 0 0 0 0 MORTALITIES: 0 1 29 40 40 0 0 0 SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 21.00

VAR OF LN OF EST. : .18672D-02

95% LOWER CONFIDENCE: 19.26 95% UPPER CONFIDENCE: 22.89

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE:

TEST NUMBER: INH-MAS

DURATION: 4 D CHEMICAL: 2-AAF SPECIES: XENOPUS

RAW DATA:

CONCENTRATION (MG/L ) 57.00 110.00 125.00 150.00 125.00 .00 .00 NUMBER EXPOSED: 40 40 40 40 0 0 0 0 MORTALITIES: 0 2 7 40 40 0 0 0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: INH.MAS 130.67

VAR OF LN OF EST. : .26987D-03

95% LOWER CONFIDENCE: 126.45 95% UPPER CONFIDENCE: 135.04

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE:

TEST NUMBER: ARO-MAS

DURATION: 4 D CHEMICAL: 2-AAF SPECIES: XENOPUS

RAW DATA:

CONCENTRATION(MG/L ) 3.00 9.60 11.00 30.00 125.00 .00 .00

NUMBER EXPOSED: 40 40 40 40 0 0 0

MORTALITIES: 0 1 6 40 40 0 0 0 0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 21.96

VAR OF LN OF EST. : .12918D-02

95% LOWER CONFIDENCE: 20.43 95% UPPER CONFIDENCE:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE:

TEST NUMBER: PB/INH/BNF-MAS

DURATION: 4 D CHEMICAL: 2-AAF SPECIES: XENOPUS

RAW DATA:

CONCENTRATION(MG/L ) 3.00 9.60 11.00 30.00 35.00 50.00 .00 NUMBER EXPOSED: 40 40 40 40 40 40 0 0 0 MORTALITIES: 0 5 8 28 38 40 0 0 0 SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 18.01 M/Y -M/S

VAR OF LN OF EST. : .42881D-02

EST. : .42881D-02 95% LOWER CONFIDENCE: 15.80 20.53

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE:

TEST NUMBER: UNACT DURATION: 4 D CHEMICAL: 2-AAF SPECIES: XENOPUS

RAW DATA:

CONCENTRATION(MG/L ) 50.00 57.00 110.00 125.00 35.00 50.00 .00 .

NUMBER EXPOSED: 40 40 40 40 40 0 0 0

MORTALITIES: 0 4 22 40 38 40 0 0 0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 90.83

VAR OF LN OF EST. : .13035D-02

95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE: 97.64

X 100 = \_\_

 $X 100 = _{.}$ 

%

%

%

X 100 = ...

5.5 mg/L

2500 mg/L

FETAX MORTALITY DATA

1

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FETAX MORTALITY DATA

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FETAX MORTALITY DATA

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				Test N	Test Number					
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FETAX MALFORMATION DATA

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FETAX MALFORMATION DATA

Investigator		
Date		
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Gut		
Edema		
Multiple		
Cardiac		
Abdominal		
Facial		
Cephalic		
Optic		
Tall		
Notochord		
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Face		
Eye		
Brain		
Hemorrhage		
Cardiac		
Bilsters		
ت ا		
No. Malformed		
Total No.	10 10 10 10 10 10 10 10 10 10 10 10 10 1	3922
Comments:		39 82

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 4/3/97 TEST NUMBER: 1 DURATION: 4 D

CHEMICAL: TCE AROCLOR

SPECIES: FETAX

RAW DATA:

SPEARMAN-KARBER ESTIMATES: LC50: 378.13

VAR OF LN OF EST. : .29384D-02

95% LOWER CONFIDENCE: 339.28 95% UPPER CONFIDENCE: 421.43

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

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HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 4/3/98
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: TCE INH
SPECIES: FETAX

RAW DATA:

SPEARMAN-KARBER TRIM:

.00%

SPEARMAN-KARBER ESTIMATES: LC50: 190.35

VAR OF LN OF EST. : .41661D-02

95% LOWER CONFIDENCE: 167.30 95% UPPER CONFIDENCE: 216.58

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 4/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: TCE B-NF
SPECIES: FETAX

RAW DATA:

SPEARMAN-KARBER ESTIMATES: LC50: 390.13

VAR OF LN OF EST. : .33159D-02

ST.: .33159D-UZ 95% LOWER CONFIDENCE: 347.69 95% UPPER CONFIDENCE: 437.75

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 4/3/97 TEST NUMBER: 1 DURATION: 4 D CHEMICAL: TCE PB SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 152.00 490.00 600.00 600.00 .00 .00 .00 NUMBER EXPOSED: 40 40 40 40 0 0 0 0 0 0 0 MORTALITIES: 0 16 40 40 0 0 0 0 0 0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 412.01

VAR OF LN OF EST. : .28279D-02

95% LOWER CONFIDENCE: 370.44 95% UPPER CONFIDENCE: 458.25

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 4/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: TCE
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 50.00 85.00 97.00 152.00 490.00 .00 .00 NUMBER EXPOSED: 40 40 40 40 40 0 0 0 0 0 MORTALITIES: 0 1 5 18 40 0 0 0 0 0 SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 181.29

VAR OF LN OF EST. : .43559D-02

95% LOWER CONFIDENCE: 158.87 95% UPPER CONFIDENCE: 206.87

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 4/3/97 TEST NUMBER: 1 DURATION: 4 D

CHEMICAL: TCE AROCLOR

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 5.00 15.00 50.00 85.00 490.00 .00 .00 NUMBER EXPOSED: 40 40 40 40 0 0 0 0 MORTALITIES: 0 1 21 40 40 0 0 0 0 SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 40.17

VAR OF LN OF EST. : .54973D-02

95% LOWER CONFIDENCE: 34.64 95% UPPER CONFIDENCE: 46.60

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 4/3/97
TEST NUMBER: 1

DURATION: 4 D CHEMICAL: TCE INH SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 5.00 16.00 50.00 85.00 490.00 .00 .00 NUMBER EXPOSED: 40 40 40 40 40 0 0 0 0 0 MORTALITIES: 10 23 40 40 40 0 0 0 0

SPEARMAN-KARBER TRIM:

25.00%

SPEARMAN-KARBER ESTIMATES: LC50: 11.90

VAR OF LN OF EST. : .28590D-01

95% LOWER CONFIDENCE: 8.49 95% UPPER CONFIDENCE: 16.69

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 4/3/97 TEST NUMBER: 1 DURATION: 4 D CHEMICAL: TCE B-NF SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 5.00 16.00 50.00 85.00 490.00 .00 .00

NUMBER EXPOSED: 40 40 40 40 0 0 0

MORTALITIES: 0 2 21 40 40 0 0 0 0

.00% SPEARMAN-KARBER TRIM:

39.70 SPEARMAN-KARBER ESTIMATES: EC50:

VAR OF LN OF EST. : .59211D-02

95% LOWER CONFIDENCE: 34.04 95% UPPER CONFIDENCE:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

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HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 4/3/97 TEST NUMBER: 1 DURATION: 4 D CHEMICAL: TCE SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 5.00 16.00 50.00 85.00 490.00 .00 .00 NUMBER EXPOSED: 40 40 40 40 40 0 0 0 0 MORTALITIES: 3 15 40 40 40 0 0 0 0

7.50% SPEARMAN-KARBER TRIM:

SPEARMAN-KARBER ESTIMATES: EC50: 18.02

VAR OF LN OF EST. : .11409D-01

95% LOWER CONFIDENCE: 14.56 95% UPPER CONFIDENCE:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 4/3/97 TEST NUMBER: 1 DURATION: 39 10 CHEMICAL: TCE MIX SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 5.00 16.00 50.00 85.00 490.00 .00 .00 NUMBER EXPOSED: 40 40 40 40 0 0 0 0 MORTALITIES: 10 39 40 40 40 0 0 0 0 SPEARMAN-KARBER TRIM: 25.00%

SPEARMAN-KARBER ESTIMATES: EC50:

VAR OF LN OF EST. : .53655D-02

95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE: 8.65

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719;

DATE: 4/3/97 TEST NUMBER: 1 DURATION: 4 D CHEMICAL: TCE SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 5.00 16.00 50.00 85.00 490.00 .00 .00 NUMBER EXPOSED: 40 40 40 40 40 0 0 0 0 0 MORTALITIES: 0 1 21 40 40 0 0 0 0 .00% SPEARMAN-KARBER TRIM:

SPEARMAN-KARBER ESTIMATES: EC50: 40.86

VAR OF LN OF EST. : .51548D-02

95% LOWER CONFIDENCE: 95% LOWER CONFIDENCE: 35.39 95% UPPER CONFIDENCE: 47.17

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 4/3 TEST NUMBER: 1 DURATION: 4 D CHEMICAL: TCE SPECIES: FETAX

RAW DATA:

CONCENTRATION (MG/L ) 152.00 490.00 600.00 85.00 490.00 .00 .00 0 0 NUMBER EXPOSED: 40 40 40 40 0 0 0 MORTALITIES: 0 16 40 40 40 0 0 0 .00%

SPEARMAN-KARBER TRIM:

SPEARMAN-KARBER ESTIMATES: LC50: 412.01

VAR OF LN OF EST. : .28279D-02

95% LOWER CONFIDENCE: 370.44 95% UPPER CONFIDENCE: 458.25

 $X 100 = _{-}$ 

X 100 =

%

%

X 100 = .

X 100 =

%

5.5 mg/L

2500 mg/L

FETAX MALFORMATION DATA

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Severe			1	<del>"</del>			<del>- ;</del>	11	1			<u> </u>	O	)   A	20 05	7	গ্ৰ	٤	5	5	9	200	2گر	TOT	AL.
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Cardlac					+	<u> </u>	+		1		Ť	Ť	$\dot{\top}$	$\frac{\perp}{1}$	<u> </u>	_	4	4			1	-			
Abdominal				+	<del> </del>	$\frac{1}{1}$	<u> </u>	1			Ť	十	i	+	$\dashv$	$\dashv$	$\downarrow$	$\downarrow$			7				
Facial			T	$\dagger$	1	+	<u> </u>	1			Ť			$\frac{1}{1}$	+	-	_	_		Ì					
Cephalle			Ī	+	1	<del> </del>	1	+			Ť	<del> </del>	$\frac{1}{1}$	$\frac{\perp}{1}$	+	<u> </u>	$\downarrow$	_			i				
Optic			Γ			$\frac{1}{1}$	+	1			1	$\dagger$	╁	+	+	+	$\downarrow$	$\bot$					_		
Tall			Ī	T		+	+	_	1		Ť	Ť	$\dot{\top}$	$\frac{1}{1}$	+	$\frac{\perp}{}$	1	_			i				
Notochord				$\dagger$	+	<u> </u> 	$\frac{1}{1}$	<u> </u>			Ť	Ť	$\frac{1}{1}$	$\frac{+}{1}$	1	$\dashv$	_	_			1				
Fla				$\dagger$		$\frac{1}{1}$	+	_	_		1	$\dagger$	╁	+	+	-	_	_							
Face				+	+	1	+	$\downarrow$			Ť	$\dagger$	$^{+}$	$\frac{+}{1}$	+	$\frac{1}{1}$	4	_			i				
Eye				<del>i</del>	<del> </del>	<u> </u> 	_	+			Ť	Ť	┪	$\frac{+}{1}$	+	$\dashv$	4	_							
Brain				1	+	+	_	_		I	Ť	$\dagger$	$\dagger$		+	+	_	$\perp$			1	+	_		
Hemorrhage			T		+	+	+-	_	1		İ	十	+	+	+	+	_	1					_		
Cardlac			T	T	+	1	+	+	1		Ť	<del> </del>	$\dagger$	$\frac{1}{1}$	+	$\frac{1}{1}$	_	_			1		-		
Bilsters				-	<del> </del>	+	+	$\bot$			Ì	+	+	$\frac{1}{1}$	$\frac{1}{1}$	+	_	4			i		<u> </u> 		
Other-specify			T	$\dagger$	+	+	+	$\downarrow$			Ť	Ť	+	+	+	+	$\downarrow$	_			ij				
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Total No.	39	38	40	~ 2	100			3			_	_			_		1	_	4	0].		7		- [	73
Comments:					H		l					2		2		100	Y	_	4040	5	9	<b>各</b>	7	ತ್ತು M	23

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TCE	6	(	96 hr																							
Test Material	Test Number		72 hr	_					_																	 
Test N	Test N		48 hr						_										1							
			24 hr																		-					 
			(X (ml)																							
			FETAX																							
Fort			Stock (ml)																							
Investigator	Date		Concentration	MIXED MAS	(F++		₹ <del>₹</del>	产于一	5	اء	20	85	9.7	152	440	909		5	9/	50	85	9.7	152	440	600	

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17/1	) 4	96 hr									-	-														<b>-</b> -
aterial	umber	72 hr																								_
Test Material	Test Number	48 hr																	-				-			
		24 hr																								
		FETAX (ml)															3									
4~		Stock (ml)																								
Investigator	Date	Concentration	FETAK	FETAX	Arouby MAS	+00	7	2	2	50	85	97	152	490	600	INH-WAS	をキャ	AH-	ρ,	ا اه	5.	85	97	152	490	

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Test Material	umber		72 hr		 							<b>-</b> -						1							1	-	_
Test M	Test Number		48 hr		-	-	1					_						-			-						_
			24 hr												-				_					-			
			FETAX (ml)																								
Fort			Stock (ml)																								
Investigator	Date		Concentration	B-NF MAS	G +	C1-	5	9	8	85	97	152	490	8	PB-MAS	(p+	<i>-</i> 67	5	٩	50	\$2	97	152	1,90	600		

# FETAX MALFORMATION DATA

Investigator	
Date	
	Test Number
TATA COLUMN	P.B. CONCENTIBATION
MALFORNIA I ION	15 15 15 15 15 15 15 15 15 15 15 15 15 1
Severe	
Stunted	
Gut	
Edema	
Multiple	
Cardiac	
Abdominal	
lincial	
Cephallo	
Optic	
Tail	
Notochord	
Fln	
Face	
Eye	
Brain	
Hemorrhage	
Cardiac	
Blisters	
Other-specify	
No. Malformed	26
	36 40 40 40 40 3917 6 6 32 23 11 36 40 39 31 30
Commen(s:	ab (52   22   12   12   12   12   12   12

## FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 5/5/97
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: TCE
SPECIES: FETAX

## RAW DATA:

CONCENTRATION(MG/L ) 97.00 152.00 490.00 600.00 .00 .00 .00

NUMBER EXPOSED: 40 40 40 40 0 0 0 0

MORTALITIES: 0 2 17 40 0 0 0 0 0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 388.93

VAR OF LN OF EST. : .36583D-02

95% LOWER CONFIDENCE:344.6295% UPPER CONFIDENCE:438.94

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

## FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 5/5/97
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: TCE PB
SPECIES: FETAX

## RAW DATA:

CONCENTRATION(MG/L ) 97.00 152.00 490.00 600.00 .00 .00 .00 NUMBER EXPOSED: 40 40 40 0 0 0 0 0 0 MORTALITIES: 0 1 15 40 0 0 0 0 0

SPEARMAN-KARBER TRIM:

.00%

SPEARMAN-KARBER ESTIMATES: LC50:

VAR OF LN OF EST. : .31613D-02

EST. : .31613D-02 95% LOWER CONFIDENCE:

367.06

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

CORRECTION 12(4):417 (1978).

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719;

DATE: 5/5/97 TEST NUMBER: 1 DURATION: 4 D CHEMICAL: TCE MIX

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 50.00 85.00 97.00 152.00 490.00 .00 .00 NUMBER EXPOSED: 40 40 40 40 0 0 0 0 0 MORTALITIES: 0 1 9 20 40 0 0 0 0

SPEARMAN-KARBER TRIM: . 00왕

SPEARMAN-KARBER ESTIMATES: LC50: 169.11

VAR OF LN OF EST. : .45342D-02

95% LOWER CONFIDENCE: 147.80 95% UPPER CONFIDENCE: 193.49

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 5/5/97 TEST NUMBER: 2 DURATION: 4 D

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 5/5/97 TEST NUMBER: 2 DURATION: 4 D

CHEMICAL: TCE AROCLOR

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 97.00 152.00 490.00 600.00 .00 .00 .00 NUMBER EXPOSED: 40 40 40 40 0 0 0 0 0 0 MORTALITIES: 0 1 19 40 0 0 0 0 0 SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 383.49

VAR OF LN OF EST. : .33380D-02

95% LOWER CONFIDENCE: 341.64 95% UPPER CONFIDENCE: 430.47

## TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

## FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 5/7/97
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: FETAX
SPECIES: TCE

## RAW DATA:

CONCENTRATION(MG/L ) 85.00 97.00 152.00 490.00 .00 .00 .00 . NUMBER EXPOSED: 40 40 40 0 0 0 0 0 0 MORTALITIES: 0 8 19 40 0 0 0 0 0

SPEARMAN-KARBER TRIM:

. 00왕

SPEARMAN-KARBER ESTIMATES: LC50: 175.27

VAR OF LN OF EST. : .44267D-02

EST.: .44267D-02 95% LOWER CONFIDENCE: 153.43 \_\_\_\_\_\_\_ CHEMICAL: TCE SPECIES: FETAX

RAW DATA:

AW DATA:

CONCENTRATION(MG/L ) 152.00 490.00 600.00 152.00 490.00 .00 .00 .

NUMBER EXPOSED: 40 40 40 40 0 0 0 0

MORTALITIES: 0 14 40 20 40 0 0 0 0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 426.40

VAR OF LN OF EST. : .26806D-02

ST.: .26806D-02 95% LOWER CONFIDENCE: 384.46 472.92

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 5/6/97 TEST NUMBER: 2 DURATION: 4 D

CHEMICAL: TCE INH

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 5.00 16.00 50.00 85.00 490.00 .00 .00 NUMBER EXPOSED: 40 40 40 40 40 0 0 0 0 0 MORTALITIES: 8 25 40 40 40 0 0 0 0 0 SPEARMAN-KARBER TRIM: 20.00%

SPEARMAN-KARBER ESTIMATES: EC50: 11.45

VAR OF LN OF EST. : .19686D-01

95% LOWER CONFIDENCE: 8.65 95% UPPER CONFIDENCE: 15.16

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 5/6/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: TCE aN
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 5.00 16.00 50.00 85.00 490.00 .00 .00 NUMBER EXPOSED: 40 40 40 40 40 0 0 0 0 MORTALITIES: 0 3 19 40 40 0 0 0 0 SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 40.22

VAR OF LN OF EST. : .66460D-02

95% LOWER CONFIDENCE:34.1795% UPPER CONFIDENCE:47.34

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 5/6
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: FETAX
SPECIES: TCE

RAW DATA:

CONCENTRATION(MG/L ) 5.00 16.00 50.00 85.00 490.00 .00 .00 NUMBER EXPOSED: 40 40 40 40 40 0 0 0 0 MORTALITIES: 0 13 40 40 40 0 0 0 0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 19.46

VAR OF LN OF EST. : .72694D-02

95% LOWER CONFIDENCE: 16.41 95% UPPER CONFIDENCE: 23.07

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 5/6/97 TEST NUMBER: 2 DURATION: 4 D

CHEMICAL: TCE NO MAS

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 5.00 16.00 50.00 85.00 .00 .00 .00 .

NUMBER EXPOSED: 40 40 40 0 0 0 0 0

MORTALITIES: 0 3 24 40 0 0 0 0 0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 36.23

VAR OF LN OF EST. : .64825D-02

95% LOWER CONFIDENCE: 30.84 95% UPPER CONFIDENCE: 42.56

																	1				
UNACTIVATED STORE COLUMNATION EX	<u> </u>	5 6.560 1.440	6.400	6.240		5.760	5.600	4.360 3.040 4.800 3.200	3.200	1.600	0000	<b>!</b>			x 2 disnes / 7.76 x 4 days 311.04		2.35 m 11 11 61	11.5.5 × 11.		C. C. C. C. C. C. C. C. C. C. C. C. C. C	0
UNACTIV Concent	(mgl/l) FX Control 5.5 6-AN	0.045	0.05	0.065	0.065	0.07	0.07		0.15	0.2	0.25										
U ACTI ATED 2-AAF	FK grittol FK grittol .5 g.N	0.02 7.3.0 0 540	7.2 0 0 000	0.00 0 0.00 0.00 0.00 0 0.00 0.00 0.00 0 0.00	6.70	6.50 11440			0.15 3.2.0 4.8.10	-(2) +(6, 50	025 0.0.0	S 1		Sum 33.280	• •		0-45 m/m/x 5000		Start Start	The state of the s	
UNACTIVATED Trichlorethylane Concentration FX Stock	(mg/mL) (mL) FX Control 5.5 & AN 2500 & AN	7.533	0.04 7.467 0.533	7.333		0.06 <b>7.</b> 200 0.800 0.065 7.133 0.867		3.333		2.000		0.667	0.00 8.000	Sum 46.667 x 2 dishes 93.33333			1 2 2 2 1 1 1 1 2 2	- 300 mg	b		
G E	(mg/mL) (mL) FX Control 5.5 6-AN 2500 6-AN	5 4.706	4 4.235 3.765 4.5 3.765 4.235	3.294		6.5 1.882 6.118	1.412		0.471	8.5 0.000 8.000			x 2 dishes 124,2353 x 4 days 496,9412	8.5 m/m2 x 300m	Ď	= 4,350 g	4				

Underty dated Definitive Paviz text 2 14 F

FX Stock MAS Generator Enzyme (mL) (mL) (mL) (mL) (mL) 8  6.933 1.067 6.667 1.333 6.400 1.600 6.133 1.867 5.867 2.133 5.600 2.400 5.333 2.667 3.200 4.800 2.933 5.067 2.667 5.333 1.33 6.667 0.000 8.000	٦	JNACTIVA	UNACTIVATED 2-AAF DEFINITIVE	DEFINITIV	ш		
(mL) (mL) (mL) (mL) (mL) (mL) (mL) (mL)	Concentration	FX	Stock	MAS	Generator	Enzyme	
8 6.933 6.667 6.400 6.133 5.867 5.333 3.200 2.933 1.333 0.000	L)	(mL)	(mL)	(mL)	(mL)	(mL)	Other
6.933 6.667 6.400 6.133 5.867 5.333 3.200 2.933 1.333 0.000	trols	8					
6.933 6.667 6.400 6.133 5.867 5.867 5.333 2.933 1.333 0.000	Control						
6.933 6.667 6.400 6.133 5.867 5.333 3.200 2.933 1.333 0.000	Control						
6.667 6.400 6.133 5.867 5.333 3.200 2.933 1.333 0.000	2	6.933	1.067				
6.400 6.133 5.867 5.333 3.200 2.933 1.333 0.000	55	6.667	1.333				
6.133 5.867 5.600 5.333 3.200 2.933 1.333 0.000	က	6.400	1.600				
5.867 5.600 3.200 2.933 1.333 0.000	35	6.133	1.867				
5.600 5.333 3.200 2.933 2.667 1.333 0.000	4	2.867	2.133				
5.333 3.200 2.933 2.667 1.333 0.000	15	5.600	2.400				
3.200 2.933 2.667 1.333 0.000	5	5.333	2.667				
2.933 2.667 1.333 0.000	6	3.200	4.800				
2.667 1.333 0.000	<del>3</del> 5	2.933	2.067				
1.333 0.000	_	2.667	5.333				
0.000	25	1.333	6.667				
	2	0.000	8.000				

## Thalidomide Mestraffis

	THIOACE	TAMIDE UN	THIOACETAMIDE UNACTIVATED RANGE	RANGE			
Concentration	Ϋ́	Stock A	Stock B	MAS	Generator Enzyme	Enzyme	
(mg/mL)	(mľ.)	(mL)	(mL)	(mL)	(mL)	(mL)	Other
FX Controls	8						
5.5 6-AN Control							
2500 6-AN Control							
0.005	7.920		0.080				
0.01	7.840		0.160				
0.05	7.200		0.800				
0.1	6.400		1.600				
0.5	0.000		8.000				
-	7.680	0.320					
2.5	7.200	0.800					
S	6.400	1.600					
10	4.800	3.200					
25	0.000	8.000					

Stock solution A=pmg/mL
Total volume of stock A required to finish one (1) test=

111.36 mL

Stock solution B=mg/mL Total volume of stock B required to finish one (1) test=85.12

# 25 =5/=1 x 200ml = 5000mg

	FE	TAX SUM	лаку	SHEE	T		<u> </u>	
							Test No.	
Test Mate	rial (M	chphoso	ham	i'de		Invest	igator 5	+
Source	5 ign	~ Chen	in	?		Lab	SA	
CAS No.	0	Lot	No.			Test St	tart Date	
Composition	on/Purity					Test En	nd Date	
Solvent		Co	nc.			Test Un	nits (i.e., mg/ml)	malmL
		DAY 1	T DA	Y 2	DA	V 3	DAY 4	DAY 5
	H	DAII	50		DA	1 3	DATA	DAIS
Stock			ļ					
Contro	i							
Highes	t Conc.							
	X CONTROL =		i		ALITY		MALFOR	
	sd or Malformed otal Number	- X 100 = %						
			0	: <u>80</u> x	100 = _	<u>O_</u> %	<u>0:80 x</u>	100 = 0 %
Solvent Cor					100 =		:x	100 =%
Control Len	igth m	m	Solve	nt Contr	ol Leng	th	mm	
Minimum C	oncentration to	Inhibit Growt	h (MCI	G)				
	MA", B-D		_					
TEST MA	TERIAL/CO	OMPOUND	: RES	SULTS				
TEST	MORTA	LITY N	1ALFO	RMATIO	N	STAT	ISTICAL TEST	USED
NOEL								
LOEL								
LC50 > 4.0,	3.09, >4.0, 1	، ا ر1،1 ره،	34	EC <sub>50</sub>	> 4.0, 1	4 < را8،	40,0.59,0,	61,0.40
95% Confide	ence limits HD, 2-1.8	3.c-3.17 5.NE	> ·!				ts ND, 172-1.9 3-0.70, 0, 3-	
T	EST TERATO	GENIC INDEX	(TI =				71, NP, 8,54	
				-				
POSITIVE	CONTROL	: 6 AMINO	NICO.	TINAN	IIDE (	(6-AN)	) RESULTS	
CONCE	VTDATION		MODT	TITV			MALEODM	710

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	:X 100 =%	:X 100 =%
2500 mg/L	:X 100 =%	:X 100 =%

FETAX MORTALITY DATA

No %	0	0	<u>ق</u>	0	0	0	0	9								-	1	5		 C	0	0	0	0	00m2
Total	010	0 0	0 1 0	0 0	0 0	0	0	0	1		<b>-</b>		<b></b>	-		-	0 1 1	3 2		0	0			0 0	004 m 6
96 hr				-																					
72 hr							•		•				1	-											
-																									
FETAX (																									
Stock (ml)																									
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	ilon Stock (ml) FETAX (ml) 24 hr 48 hr 72 hr 96 hr Total No		on         Stock (ml)         FETAX (ml)         24 hr         48 hr         72 hr         96 hr         Total         No           1         1         1         1         0         0         0           2         1         1         1         0         0         0	Slock (ml) FETAX (ml) 24 hr 48 hr 72 hr 96 hr Total No	Stock (ml) FETAX (ml) 24 hr 48 hr 72 hr 96 hr Total No   Stock (ml) A8 hr 72 hr 96 hr Total No   Stock (ml) A8 hr 72 hr 96 hr Total No   Stock (ml) A8 hr 72 hr 96 hr Total No   Stock (ml)	Ilon         Stock (ml)         FETAX (ml)         24 hr         48 hr         72 hr         96 hr         Total         No           S <td>Ilon         Stock (ml)         FETAX (ml)         24 hr         48 hr         72 hr         96 hr         Total         No           S<td>Ilon         Stock (ml)         FETAX (ml)         24 hr         48 hr         72 hr         96 hr         Total         No           A         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Severe							-						-	-	<u>"</u>	i	<u> </u>			-				
Stunted		<u> </u>								İ				<del> </del>		-	<u> </u>	-	-	<u> </u>	_	<u> </u>		
Gut		_		-	_	_	_			İ	İ	$\dot{\parallel}$	-	-	$\vdash$	-	$\vdash$	-	-	-	_	_		
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Optic					_								<del> </del>	-	+	<del> </del>	-	-	<u> </u>	-	_	_		
Tall													<u> </u>	$\frac{1}{1}$	<del> </del>	-	-	<del>!    </del>	<u> </u>	<u> </u>	<u>                                     </u>	_		
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Brain														<del>                                     </del>	$\vdash$	-	-	-	<u> </u>	_	-	_	<u> </u>	
Hemorrhage															-					-	-	-	_	
Cardiac																<del>                                     </del>	-	$\vdash$	<del> </del>	_	_	_	_	
Blisters		_											İ	$\vdash$		$\vdash$		-	<u>                                       </u>	-	<del> </del>	<u> </u>	_	
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Total No.	an Can	2000	0 31	1 33	3 9	7	1	1		3	3	38	22	6	8	13	<u>۔</u> عو	3	Ze 39					
Comments:				77							41						-#	→+		71		_11		

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 12/4/96 TEST NUMBER: 1 DURATION: 4 D CHEMICAL: CPA B-NF SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML ) 2.00 2.50 3.00 3.50 4.00 .00 .00 NUMBER EXPOSED: 40 40 40 40 40 0 0 0 0 MORTALITIES: 0 3 8 40 40 0 0 0 SPEARMAN-KARBER TRIM: .00% .00%

SPEARMAN-KARBER ESTIMATES: LC50: 3.09

VAR OF LN OF EST. : .18450D-03

95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.

ENVIRON. SCI. TECHNOL. 11(7): 714-719;

CORRECTION 12(4):417 (1978).

DATE: 12/4 TEST NUMBER: 1 DURATION: 4 D CHEMICAL: CP INH SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML ) 2.00 2.50 3.00 3.50 4.00 .00 .00 NUMBER EXPOSED: 40 40 40 40 40 0 0 0 0 MORTALITIES: 0 3 8 27 38 0 0 0

SPEARMAN-KARBER TRIM:

5.00%

SPEARMAN-KARBER ESTIMATES: LC50: 3.28

VAR OF LN OF EST. : .33661D-03

95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 12/4/96 TEST NUMBER: 1 DURATION: 4 D

CHEMICAL: CPA AROCLOR

SPECIES: FETAX

RAW DATA:

CONCENTRATION (MG/ML ) .75 1.00 1.25 1.50 2.00 2.50 .00

NUMBER EXPOSED: 40 40 40 40 40 0 0

MORTALITIES: 0 3 7 21 35 40 0 0

.00% SPEARMAN-KARBER TRIM:

SPEARMAN-KARBER ESTIMATES: LC50: 1.50

VAR OF LN OF EST. : .78417D-03

95% LOWER CONFIDENCE: 1.42 95% UPPER CONFIDENCE:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 12/4 TEST NUMBER: 1 DURATION: 4 D CHEMICAL: CPA - PB

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML ) .75 1.00 1.25 1.50 2.00 2.50 .00 NUMBER EXPOSED: 40 40 40 40 40 0 0 0 MORTALITIES: 0 1 7 13 24 40 0 0 0

.00% SPEARMAN-KARBER TRIM:

SPEARMAN-KARBER ESTIMATES: LC50: 1.70

VAR OF LN OF EST. : .88239D-03

95% LOWER CONFIDENCE:

95% UPPER CONFIDENCE: 1.81

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.

ENVIRON. SCI. TECHNOL. 11(7): 714-719;

CORRECTION 12(4):417 (1978).

DATE: 12/4 TEST NUMBER: 1 DURATION: 4 D CHEMICAL: CPA SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L (M) .50 .75 1.00 1.25 1.50 2.00 .00 NUMBER EXPOSED: 40 40 40 40 40 0 0 0 MORTALITIES: 0 2 8 11 22 40 0 0 0 SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 1.34

VAR OF LN OF EST. : .95015D-03

95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.

ENVIRON. SCI. TECHNOL. 11(7): 714-719;

CORRECTION 12(4):417 (1978).

DATE: 12/4 TEST NUMBER: 1 DURATION: 4 D

CHEMICAL: CPA - BNF

SPECIES: FETAX

RAW DATA:

CONCENTRATION (MG/ML ) 1.00 1.25 1.50 2.00 2.50 2.00 .00 NUMBER EXPOSED: 40 40 40 40 37 40 0 0 0 MORTALITIES: 0 1 10 23 37 40 0 0 0

.00% SPEARMAN-KARBER TRIM:

SPEARMAN-KARBER ESTIMATES: EC50: 1.81

VAR OF LN OF EST. : .68247D-03

95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 12/4 TEST NUMBER: 1 DURATION: 4 D CHEMICAL: CPA - INH

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML ) 1.50 2.00 2.50 3.00 3.50 2.00 .00 NUMBER EXPOSED: 40 40 37 32 13 40 0 0 MORTALITIES: 0 1 5 19 13 40 0 0 SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50:

VAR OF LN OF EST. : .38293D-03

95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE:

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 12/4 TEST NUMBER: 1 DURATION: 4 D

CHEMICAL: CPA - AROCLOR

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML ) .10 .50 .75 1.00 1.25 2.00 .00 .

NUMBER EXPOSED: 40 40 40 37 35 40 0 0 0

MORTALITIES: 0 8 23 35 35 40 0 0 0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: .59

VAR OF LN OF EST. : .48838D-02

95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE:

FE'	TAX SUM	MARY SHEE	T							
					Test No.	2				
Test Material ( yc	lophosph	innide		Investiga	tor For	+				
Source Signa	lophosph	uQ		Lab	SIA					
CAS No.		No.		Test Star	t Date					
Composition/Purity				Test End (	Date					
Solvent	Co	onc.		Test Unit:	s (i.e., mg/ml)	mg/mL				
pH	DAY 1	DAY 2	DAY.	3	DAY 4	DAY 5				
Stock										
Control										
Highest Conc.										
FETAX CONTROL =		MORT	ALITY		MALFOR	MATION				
No. Dead or Malformed		44	CORD		REC					
Total Number	- X 100 = %									
		<u> </u>	100 = 2	18 _	<u>2 : 78 х</u>	100 =%				
Solvent Control MAS	5	0:40 x	100 = _0	_%	3 : <u>40</u> x	100 = 7.5%				
Control Length m	m	Solvent Contr	ol Length		mm					
Minimum Concentration to										
Minimum Concentration to Inhibit Growth (MCIG)  Wastiveto / B-NF/INH / Aroclar/PB/Mix										
TEST MATERIAL/CO										
TEST MORTA	LITY	MALFORMATIO	N	STATIS	STICAL TEST	USED				
NOEL										
LOEL										
LC50 >4,00/3,13/>4,00	/1.85/1.59	/1,69 EC50	>4,00/1.	86/74	, 0/0.71/0	1.92/0.72				
95% Confidence limits	0-3,28/ND/1 -1,67/1,58-1	1,74-1,98 95% C	Confidence			0,63-0.81				
		$K (TI = LC_{50} / I$	EC <sub>50</sub> )	,	8/ND/2.61/1	1				
POSITIVE CONTROL	.: 6 AMINO	NICOTINAN	AIDE (6	-AN)	RESULTS					
				-						

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	:X 100 =%	:X 100 =%
2500 mg/L	:X 100 =%	:X 100 =%

FETAX MORTALITY DATA

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	aterial	umber		72 hr			-	1-			_			-			-			-		-		-	 			-
	Test Material	Test Number		48 hr	-			-															-		 			
			4.5.5	74 hr			_	-	+	1	1	1	-	1							1	-		-	 			
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T T		12/30/9/0	Stock (ml)																									
Investigator			Concentration	16 TA		XX	CKK	5	15		0.1	5.0	27.0		1.25		7	2.5	3	2.5	2							

Investigator	Fort	+										Ţ	Me	Test Material		5	7							Į.
Date	20	1	2								j					1		PN	12	1	- MAS	5		
	3		16/02									l es	i est Number	nber		2								
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Multiple	-	+-	<u> </u> 	+	+	1			$\dagger$	-	+	+	-	İ	+	+	+	4			1	$\dashv$		
Cardiac		<del>                                     </del>		<u> </u> 	<u> </u>	_			İ	+	$\frac{1}{1}$	+	1	İ	<del> </del>	+	+	1		Ì	$\dashv$			
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Facial	<del> </del>	<u> </u>	<u> </u>	<u> </u>	+	1	1		1		$\frac{1}{1}$	+	+	İ	+	+	<u> </u>	_		Ì	+	1		
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Optic		-	_	-	<u> </u>		1		Ť	+	+	+	$\downarrow$	Ţ	+	+	+	-						
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Face		-	-	<u> </u>	+	$\perp$	_	I	$\dagger$	+	+	+	+	1	$\dagger$	+	+	_			1			
Eye		<del> </del>	<u> </u>	<u> </u>	<u> </u>	-	1				$\frac{\perp}{1}$	1	+	İ	$\dagger$	+	+	1		Ī	1	+		
Brain		<del> </del>	<u> </u>	<u> </u>	-	_	1		Ť	+	+	-	_			$\frac{1}{1}$	+	$\downarrow$			+	$\dashv$		
Hemorrhage		<u> </u>	1	-	<del> </del>	-	_		T	+	+	$\frac{1}{1}$	_	1	+	+	+	+			$\dagger$	+		
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Other-specify		<u> </u>	-	<del> </del>	<u> </u>	_			1	+	+	+	_	1	$\dagger$	+	+	$\downarrow$			1	$\dashv$		
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FETAX MORTALITY DATA

Investigator	4				11	Ì				
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l/A(C	8/8/90			Test Number	1	1	CANAL TANA	3		
Concentration	Stock (ml)	GCTAV /				<b>4</b>				
	(1111)	reiaa (mi)	24 hr	48 hr	72 hr	96 hr	Total	٥N	*	li
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12/3     9 L	Investigator			
Test Number   2	Dete	) col material ( )	(SAM 1+12	
RMATION   M   Cr-   0.1   0.5   0.35   1   1.33   1.5   2   2.5   3   3.5   4	DAIC	131/96 Test Number 2		
SMATION   M   Cr-   O-1   O-5   Cort   1   1-35   1-5   3   5   4				
MATION   M   Cr.   0.1 0.5 0.7 1   1.35 1.5 2 2.5 3 3.5 4				
	MALFORMATION	CP- 0.1 0.5 075 1 1.35 1.5 2 2.5 3 3.5	101	
the continal   hall c   hall	Severe			
	Stunted			
	Gut			
Ity	Edema			
Ity   Ity	Multiple			
In a	Cardiac			
11y	Abdominal			
C	Facial			
i	Cephalic			Ī
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ie  iv  iv  iv  iv  iv  iv  iv  iv  iv	Tall			
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If y   1   1   1   1   1   1   1   1   1	Eye			
11y  11y  11y  1-y  1-y  1-y  1-y  1-y	Brain			Ī
1fy or 40 0	Hemorrhage			Ī
If y    1   1   2   2   2   3   5   5   10	Cardiac			Ī
1fy or the contract of the con	Bilsters			ĺ
10 40 40 6 6 6 6 7 7 10 6 7 7 10 7 7 10 7 10 7	Other-specify			
40 HO HO 35	No. Malformed	22 0 V V V V V V V V V V V V V V V V V V		Ī
	Total No.	140 140 140 140 35		T
	Comments:			

FETAX MORTALITY DATA

Sick (ml) FETAX (ml) 24 hr 48 hr 72 hr 96	Investigator Date	For t	9		Test Material	Test Material (	M	Arocha MAS	MAS)			
3000 (ml) FETAX (ml) 24 hr 72 hr 96 hr 70 ml No 1		7 H	# #									
021 0000 T 2 2 2 3 021 0000 T 2 2 2 3	llon	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total	°Ž	*		
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DAIC	121	13.	196	0							Ţ	st N	Test Number			٦			K		2	
										CONCENTRATION	9.1.7					41				***************************************		
MALFORMATION		(0)			7	4	-		-				<u>.</u>	-		f	-	-				
Severe	-	7,			70	-	1,25	S   S	7	2:3	-	-#				Ï						TOTAL
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Multiple		<del> </del>		Ī	†	+	+	+	-	1		+-	-	+			$\dashv$	$\dashv$	$\dashv$			
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Optic		_				-	+	-	+		$\dagger$	+	+	_			+	+	-	1		
Tall		<u> </u>				+		1	1		$\dagger$	+	+	1		T	+	$\dashv$	_	$\downarrow$		
Notochord		-			İ	+	+	+	-	Ţ	$\dagger$	+	<u> </u>	1			$\frac{1}{1}$	$\dashv$	1	1		
Fin			1		Ì	+	+	+	+		$\dagger$	$\frac{1}{1}$	+	_			$\dashv$	$\dashv$	$\dashv$	$\downarrow$		
Face		-	_			$\dagger$	+	+	_		$\dagger$	-	+	1			+	$\dashv$	_	1		
Eye		<u> </u>			İ	+	+	<u> </u>	+		Ť	+	+	$\downarrow$			+	+		_		
Brain		_				$\dagger$	+	+	_	Ţ	$\dagger$	+	+	_		Ť	$\dashv$	+	$\dashv$	_		
Hemorrhage		$\perp$			Ī	+	+	+	1	Ţ	$\dagger$	+	+	1		1	$\dashv$	$\dashv$	_			
Cardlac	+	$\frac{1}{1}$	_		<u> </u>	$\dagger$	+	$\frac{1}{1}$	+	I	$\dagger$	+	+	1		T	+	+	$\frac{1}{1}$			
Blisters		+			Ť	$\frac{1}{1}$	+	+	1	$\prod$	$\dagger$	+	+	1		Í	$\dashv$		-			
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FETAX MORTALITY DATA

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Gut		$\top$	T	$\dagger$	$\dagger$		+	+	+	+	_			-	+	+	+	1		$\dashv$	+	<u> </u>	
Edema		<del> </del>	<del> </del>	$\dagger$	+	+	+	1	+	1	_			+	+	+	+	_		$\dashv$	+	_	
Multiple		T		$\dagger$	<del> </del>	+	+	+	+	+	_				+	+	$-\frac{1}{1}$	-		+		$\dashv$	
Cardiac			$\dot{T}$	$\dagger$	i	$\dagger$	十	+	+	+	$\bot$	1		+	+	+	<del> </del>	1			+	1	
Abdominal			-	†	<del> </del>	+	+	+	+	<u> </u>	_	$\perp$		+	+	+	+	_		$\dagger$		+	
Facial				Ϊ	<del> </del>	+	<del> </del> -	+	$\frac{1}{1}$	$\perp$	+	1		<del></del>	+	+	+	1	Ī	i		1	
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Optic		T		<del> </del>	$\dagger$	+	-	+	+	+	$\downarrow$	1		$\top$	+	+	+	_		$\dashv$	$\dashv$	+	
Tall			T	†	+	+	+	<u> </u> 	-	<u> </u>	1	<u> </u>		$\dagger$	+	+	$\frac{1}{1}$	1		$\dagger$	1	1	
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Face		T	Ť	$\dagger$	十	$\dagger$	+	+	+	-	+	1			+	+	+				$\dashv$		
Eye			T	İ		+	+	+	+		-	_		+	$^{+}$	╬	_	+			$\dashv$	1	
Brain		$\dot{T}$			$\dagger$	$\dagger$	+	+	+	<u> </u>	+			1	+	╀		_		$\dagger$	$\dashv$	$\dashv$	
Hemorrhage			$\vdash$			$\dagger$	+	-	+	-	-	1		Ť	+	+	+	_		1	$\dagger$	+	
Cardiac					$\dagger$	$\dagger$	+	+	+	+	$\perp$	1		İ	+	+	+	-		$\dagger$	+	$\dashv$	
Blisters			$\vdash$	$\vdash$	<del> </del>	$\dagger$	<u> </u>	+	+	-	-	1		<del></del>	+	+	+		Ì	$\dagger$	$\frac{1}{1}$		
Other-specify			T	$\vdash$	<del> </del>	<del> </del>	+	+	+	_	_			<del> </del>	+	+	+	$\perp$		+	$\dashv$	+	
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FETAX MORTALITY DATA

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FETAX MORTALITY DATA

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FETAX MALFORMATION DATA

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Edema			İ		-	+	-			Ť	+	+	1				+	-	+	+	+	_		
Multiple			T	+	-	+	-	1		$\dagger$	$\dagger$	+	+	-			1		+	+	+	$\perp$		
Cardiac			İ	<del> </del>	1	-	1			Ť	$\dagger$	$\frac{1}{1}$	+			Ī	$\dagger$	$\dagger$	+	+	+	$\downarrow$		
Abdominal			İ	+	+	-	+			T	$\dagger$	$\dagger$	+	_		T	Ť	$\dagger$	+	+	+	_		
Facial			İ	-	1	-	<u> </u>	1	Γ	İ	i	+	1	1	I		Ť	$\dagger$	+	+	+	$\downarrow$		
Cephalle			İ	<del> </del>	<u> </u>		<u> </u>		I	Ť	<del> </del>	+	+	<del> </del>	$oxed{oxed}$		Ť	$\dagger$	+	+	$\frac{1}{1}$	1		
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FETAX MALFORMATION DATA

Investigator																				
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Cardiac			<u> </u>	1		<u> </u>	İ	+	+	İ	1	1		+	+		$\dashv$	+	_	
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Facial		1	<del> </del>	1		+	İ	+	1	Ì	$\frac{\perp}{\parallel}$	1	İ	$\frac{1}{1}$	$\dashv$		1	$\dashv$		
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Optic			<u> </u> 	<del>                                     </del>		$\perp$	İ	+	1		+	1		$\frac{1}{1}$	+			$\dashv$	4	
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FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 12/31/96 TEST NUMBER: 2 DURATION: 4 D

CHEMICAL: CP B-NF MAS

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML ) 1.50 2.00 2.50 3.00 3.50 4.00 .00 NUMBER EXPOSED: 40 40 40 40 40 40 0 0 0 MORTALITIES: 0 2 6 14 21 40 0 0 0 SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 3.13

VAR OF LN OF EST. : .49844D-03

95% LOWER CONFIDENCE: 3.00 95% UPPER CONFIDENCE: 3.28

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 12/31/96 TEST NUMBER: 2 DURATION: 4 D CHEMICAL: CP SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML ) 1.00 1.25 1.50 2.00 2.50 3.00 .00 NUMBER EXPOSED: 40 40 40 38 34 26 0 0 MORTALITIES: 0 3 10 20 30 26 0 0 0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 1.86

VAR OF LN OF EST. : .88363D-03

95% LOWER CONFIDENCE: 1.75 95% UPPER CONFIDENCE: 1.97

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 12/31/96 TEST NUMBER: 2 DURATION: 4 D

CHEMICAL: CP AROCLOR-MAS

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML ) 1.00 1.25 1.50 2.00 2.50 3.00 .00 NUMBER EXPOSED: 40 40 40 40 40 40 0 0 0 MORTALITIES: 0 5 14 23 27 40 0 0 0 SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 1.85

VAR OF LN OF EST. : .10504D-02

1.74 1.98 95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 12/31/96 TEST NUMBER: 2 DURATION: 4 D

CHEMICAL: CP AROCLOR MAS

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML ) .10 .50 .75 1.00 1.25 3.00 .00 NUMBER EXPOSED: 40 40 40 40 35 40 0 0 0 MORTALITIES: 0 5 18 28 35 40 0 0 0

SPEARMAN-KARBER TRIM:

.00%

SPEARMAN-KARBER ESTIMATES: EC50:

VAR OF LN OF EST. : .38610D-02

95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 12/31/96 TEST NUMBER: 2 DURATION: 4 D

CHEMICAL: CP PB-MAS

SPECIES: FETAX

RAW DATA:

CONCENTRATION (MG/ML ) .75 1.00 1.25 1.50 2.00 2.50 .00 NUMBER EXPOSED: 40 40 40 40 40 40 0 0 0 MORTALITIES: 0 1 5 16 34 40 0 0 0 (CREARMAN MARRIED TRIM:

.00% SPEARMAN-KARBER TRIM:

SPEARMAN-KARBER ESTIMATES: LC50:

VAR OF LN OF EST. : .69143D-03

95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE: 1.67

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 12/31/96 TEST NUMBER: 2 DURATION: 4 D CHEMICAL: CP PB-MAS

SPECIES: FETAX

RAW DATA:

SPEARMAN-KARBER ESTIMATES: LC50:

VAR OF LN OF EST. : .73314D-03

95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 12/31/96 TEST NUMBER: 2 DURATION: 4 D

CHEMICAL: CP MIX-MAS

SPECIES: FETAX

RAW DATA:

CONCENTRATION (MG/ML ) .75 1.00 1.25 1.50 2.00 2.50 3.00 .

NUMBER EXPOSED: 40 40 40 40 40 40 0

MORTALITIES: 0 2 6 20 25 32 40 0 0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50:

VAR OF LN OF EST. : .11003D-02

95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714~719;

CORRECTION 12(4):417 (1978).

DATE: 12/31/96 TEST NUMBER: 2 DURATION: 4 D

CHEMICAL: CP MIX-MAS

SPECIES: FETAX

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NUMBER EXPOSED: 40 40 40 38 34 40 40 0

MORTALITIES: 0 5 16 27 34 32 40 0 0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: .72

VAR OF LN OF EST. : .38491D-02

95% LOWER CONFIDENCE: .63 95% LOWER CONFIDENCE: .03 95% UPPER CONFIDENCE: .81

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SBIR CYCLOPHOSHAMIDE PB ACT DEF. MAS

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SBIR CYCLOPHOSPHAMIDE DEF MAS BNF/INH/PBACT.

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## SBIR COUMARIN 2 MIX ACT. 1-13-97 -> C2MIXA

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### SBIR DEF. TEST COUMARIN AROACT

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SBIR DEF TEST COUMARIN MIX ACT.

-> CMIXA

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SBIR DEF. TEST
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# SBIR 2AAF MIX ACT. 1-97

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IMMMMMMMMMMMM II 111111111111		<i>имимимимимими</i>	IMMM HMM MMMMMMMMMMMMM	импинимми» Ім БОЯВОЙ миний Ан Миниминиминий	MUMMMMMMMM ESC MEN MAMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMM	imm; immč immm;
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Нипиманимининый.	имыйымымымымы	<i>MMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMMM</i>	<i>MMMMMMMMMMMM</i> tatistics	MHMMMMMMMMMM	4	'พพพ<ั

1198 Ach-1 MAS 3 ar Ar 11 1198 5 7 8 9 10 u 11

FETAX TE	ST CONCE	NTRATION SPE	READSHEET
COMPOU	ND:	COUMARIN	
EC50=	59.8		
LC50=	168.7		
EC5	5.98		
EC16	19.136		
EC50	59.8		
EC84	100.464		
EC95	113.62		
LC5	16.87		
LC16	53.984		
LC50	168.7		
LC84	283.416		
LC95	320.53		

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 11/13/96 TEST NUMBER: 1 DURATION: 4 D

CHEMICAL: CYCLOPHOSPHAMIDE

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML ) 5.50 6.00 6.50 7.00 7.50 8.00 .00 .

NUMBER EXPOSED: 40 40 40 40 40 0 0 0

MORTALITIES: 0 2 13 23 39 40 0 0 0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 6.76

VAR OF LN OF EST. : .74859D-04

95% LOWER CONFIDENCE: 6.65 95% UPPER CONFIDENCE: 6.88

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 11/13/96 TEST NUMBER: 1 DURATION: 4 D

CHEMICAL: CYCLOPHOSPHAMIDE

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML ) 3.50 4.00 4.50 5.00 5.50 .00 .00 .

NUMBER EXPOSED: 40 40 40 40 0 0 0 0

MORTALITIES: 0 1 6 22 40 0 0 0 0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 5.83

VAR OF LN OF EST. : .11159D-03

95% LOWER CONFIDENCE: 5.71 95% UPPER CONFIDENCE: 5.95

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 11/13/96 TEST NUMBER: 1 DURATION: 4 D CHEMICAL: 2-AAF SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML ) .05 .09 .10 .10 .13 .15 .00 .

NUMBER EXPOSED: 40 40 40 40 40 0 0 0

MORTALITIES: 0 1 4 8 39 40 0 0 0

SPEARMAN-KARBER TRIM: .00%

.11 SPEARMAN-KARBER ESTIMATES: LC50:

VAR OF LN OF EST. : .16937D-03

95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE:

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 11/13/96 TEST NUMBER: 1 DURATION: 4 D CHEMICAL: 2-AAF SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML ) .02 .03 .03 .04 .04 .05 .00 .

NUMBER EXPOSED: 40 40 40 40 40 0 0 0

MORTALITIES: 5 10 19 26 38 40 0 0 0

SPEARMAN-KARBER TRIM: 12.50%

SPEARMAN-KARBER ESTIMATES: EC50: .03

VAR OF LN OF EST. : .91693D-03

95% LOWER CONFIDENCE: .03 95% UPPER CONFIDENCE: .03

FOR REFERENCE, CITE: HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 11/13/96 TEST NUMBER: 1 DURATION: 4 D CHEMICAL: TCE SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML ) .35 .40 .45 .50 .55 .05 .00 .

NUMBER EXPOSED: 40 40 40 40 40 40 0 0 0

MORTALITIES: 0 3 7 13 40 40 0 0 0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: .49

VAR OF LN OF EST. : .12753D-03

95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE:

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 11/13/96 TEST NUMBER: 1 DURATION: 4 D CHEMICAL: TCE SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L ) 35.00 40.00 45.00 50.00 55.00 60.00 65.00 NUMBER EXPOSED: 40 40 40 40 40 40 0 MORTALITIES: 0 2 10 18 21 38 40 0 0 SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 50.80

VAR OF LN OF EST. : .19949D-03

95% LOWER CONFIDENCE:49.3895% UPPER CONFIDENCE:52.25

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 11/13/96
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: COUMARIN
SPECIES: FETAX

RAW DATA:

 SPEARMAN-KARBER TRIM:

.00%

SPEARMAN-KARBER ESTIMATES: LC50: **2**68.74

VAR OF LN OF EST. : .77755D-03

95% LOWER CONFIDENCE: **254.17**95% UPPER CONFIDENCE: **284.16** 

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977. TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS. ENVIRON. SCI. TECHNOL. 11(7): 714-719; CORRECTION 12(4):417 (1978).

DATE: 11/13/96 TEST NUMBER: 1 DURATION: 4 D CHEMICAL: COUMARIN

SPECIES: FETAC

RAW DATA:

CONCENTRATION(MG/L ) 50.00 55.00 60.00 65.00 70.00 60.00 65.00 .

NUMBER EXPOSED: 40 40 40 40 40 40 40 0

MORTALITIES: 0 11 19 29 40 38 40 0 0

SPEARMAN-KARBER TRIM: . 00%

SPEARMAN-KARBER ESTIMATES: EC50: 59.79

VAR OF LN OF EST. : .11453D-03

95% LOWER CONFIDENCE: 58.52 95% UPPER CONFIDENCE: 61.08

FETAX TE	ST CONCE	NTRATIC	N SPREAD	SHEET
COMPOU	ND:	2-AAF		
EC50=	110			ļ
LC50=	30			
EC5	11			
EC16	35.2			
EC50	110			
EC84	184.8			
EC95	209			
LC5	3			
LC16	9.6			
LC50	30			
LC84	50.4			
LC95	57			

EETAV TE	ST CONCE	NITRATIONS	PREADSHEET
LEIAV IE	STCONCE	NIKATIONS	PREADSHEET
COMPOU	ND:	COUMARIN	
EC50=	59.79		
LC50=	268.74		
EC5	5.979		
EC16	19.1328		
EC50	59.79		
EC84	100.4472		
EC95	113.601		
LC5	26.874		
LC16	85.9968		
LC50	268.74		
LC84	451.4832		
LC95	510.606		

FETAX TE	ST CONCE	NTRATION	SPREADSHEET
COMPOU	ND:	TCE	
T050-	400		
EC50=	490		
LC50=	51	· · · · · · · · · · · · · · · · · · ·	
F05	40		
EC5	49		
EC16	156.8		
EC50	490		
EC84	823.2		
EC95	931		
1.05			
LC5	5.1		
LC16	16.32		
LC50	51		
LC84	85.68		
LC95	96.9		

FETAX TE	ST CONCE	NTRATION	SPREADS	SHEET
COMPOU	ND:	CYCLOPH	OSPHAMIE	ĎΕ
EC50=	4.83			
LC50=	6.76			
EC5	0.483			
EC16	1.5456			
EC50	4.83			
EC84	8.1144			
EC95	9.177			
LC5	0.676			
LC16	2.1632			
LC50	6.76			
LC84	11.3568			
LC95	12.844			

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CAS No.		Lot	No.		7	Test S	tart Date	
Composition	on/Purity					Test Er	nd Date	
Solvent		Co	nc.			Test Ur	nits (i.e., mg/ml)	'mg/ml
		DAY 1	DA	Y 2	DA	Y 3	DAY 4	DAY 5
Stock	)H							
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Solvent Con			:		100 = _		:>	( 100 =%
Control Len	egth mo	1	Solve	nt Contr	oi Leng	th	mm	<del></del>
Minimum Co	oncentration to	Inhibit Growt	h (MCI	G)				
TEST MA	TERIAL/CO	MPOUND	: RES	SULTS				
TEST	MORTAL	ITY N	AALFO	RMATIO	N	STAT	ISTICAL TES	T USED
NOEL								
LOEL								
LC <sub>50</sub>	6.7	b		EC <sub>50</sub>			4: <i>83</i>	
95% Confide	ence limits b,	65-6.8	8	95% C	onfiden	ice Limi	ts 4.71-	4.95
T	EST TERATOG	ENIC INDEX	(TI =	LC 50 /E	C <sub>50</sub> )			The state of the s
POSITIVE	CONTROL:	6 AMINO	NICO.	TINAN	IIDE	(6-AN	) RESULTS	3
CONCE	NTRATION		MORTA	LITY			MALFORM	IATION
5.5 mg/L	_	0:4	⊇_X 10	0 = 0	%	24	: 46 X 100	=%

25:40 x 100 =

2500 mg/L

15: 15 x 100 = 100 %

Investigator	7,4			Test	Test Material	-				
Date	11/12/96				Test Number	de Jan	gelsphusphunge	مريد		
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Concentration	Stock (ml)	FETAX (m1)	24 hr	48 hr	72 hr	96 hr	Total	I NI		
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# FETAX MALFORMATION DATA

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MALFORMATION	FIG	P 16	£ 1.	8	7	12	1		-			\ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \		_		-	-	-	-					
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Cardiac			<u> </u>	<u>                                      </u>	<u> </u>		_		İ			+	<u> </u>	1		İ	+	+	+	-				
Abdominal		-	-	<u> </u>	<u> </u>	-			İ	$\dagger$	+	+	+	+		1	$\dagger$	+	+	$\downarrow$				
Facial		<del> </del>	<u> </u>	<del> </del>	<u> </u>					$\dagger$	+	+	1	_	I		$\frac{1}{1}$	+	+	+	_			
Cephalic		<del> </del>	<u> </u>	1	<u> </u>	1	_		T	$\dagger$	+	+	<u> </u> 	1		İ	$\dagger$	1	-	$\downarrow$	_			
Optic		-		<del> </del>	<u> </u>	-	_		T	$\dagger$	+	+	+	-		İ	$\dagger$	+	+	$\frac{1}{2}$	$\perp$			
Tall		<del> -</del>	<del> </del>	<del> </del>	<del> </del>					$\dagger$	+	+		_		İ	$\dagger$	+	+	_	1			
Notochord		十	<u>                                      </u>	<u> </u>	<del> </del>	-			Ī	$\dagger$	+	+	1	-	I	İ	-	+	+	_	1			
Fin		$\vdash$		-	+	-				+	$\dagger$	+	$\frac{\perp}{\parallel}$	+			$\dagger$	+	$\dashv$	_	4			
Face		+	+	+	_	_				十	$\dagger$	+	$\frac{1}{1}$	-	$\prod$	j	$\dashv$	+	$\dashv$	4	_			·
Eye		+	-	<del> </del>	+	+				$\dagger$	+	+	+	1	$\perp$		$\top$	+	-	$\perp$	4			
Brain		╁		+	+	_				$\dagger$	+	+	<u> </u>	+			+	+	+	$\perp$	1			
Hemorrhage		$\vdash$	+	-	-	_	_			$\dagger$	$\frac{1}{1}$	+	+	+			+	+	+	$\downarrow$				
Cardiac		<del> -</del>		-	╀	-			İ	$\dagger$	+	+	+	+			十	+	+	-	1			
Blisters		-	-	-	-	-	_			+	-	+	+	+		Ť	$\dagger$	+	+	_	1			
Other-specify		<del> -</del>		-	<u> </u>	-	1			+	$\frac{1}{1}$	+	+	-	I	Ţ	+	+	+	$\downarrow$	$\downarrow$			
No. Malformed	0	0	24	12	0	-	10	22	£	300	27	-	1	-		İ		#	-					
Total No.	40 1	40 4	70 40 15	1	1 4	4				1 %	_	1				T	$\dagger$	+	+	+				
Comments:									_,		71			_			1	-		_				

F	ETAX SUM	MARY	SHEET				
•						Test No.	1
Test Material 2 -	AAF				Invest	igator For	+
Source Sign		SR	12-An	$\sim$	Lab	A	
CAS No.		No.		1	Test S	tart Date	
Composition/Purity					Test Er	nd Date	
Solvent	Co	nc.			Test Ur	nits (i.e., mg/ml)	mg/mb
	DAY 1	DA	Y 2	DAY	' 3	DAY 4	DAY 5
— pH — Stock							
Control							
Highest Conc.							
FETAX CONTROL No. Dead or Malformed	X 100 = %		■ MORTAL RECOR			—— MALFOR REC	
Total Number		:	80 x 100	) <b>=</b> _(	<u>_</u> %	<u>0:80</u> x	100 =%
Solvent Control		<u> </u> :	X 100	) =	%	:x	100 =%
Control Length	m m	Solve	nt Control I	engt	h	mm	
Minimum Concentration	o Inhibit Growt	h (MCIO	G)				
TEST MATERIAL/C	OMPOUND	: RES	SULTS				
TEST MORT.	ALITY N	MALFOR	RMATION		STAT	ISTICAL TEST	USED
NOEL			·				
LOEL			r				
LC <sub>50</sub> O. 1	1		EC <sub>50</sub>		0.03	3	
95% Confidence limits	0.105 - 0.1	115	95% Conf	ideno	e Limi	ts 0.025-	0.035
TEST TERAT	OGENIC INDEX	(TI =	LC 50 /EC 50	)			
POSITIVE CONTRO	L: 6 AMINO	NICO	TINAMIC	)E (	6-AN	) RESULTS	
CONCENTRATION		MORTA	LITY			MALFORMA	TION

0:40 x 100 = 0 %

25: 40 x 100 = \_\_\_\_

18:40 × 100 = 45 %

15:15 x 100 = 100 %

5.5 mg/L

2500 mg/L

# FETAX MALFORMATION DATA

Investigator	L	1										Í					,								F
Dete	-	7											2   2	I COL NIBICLIBI	=		7	VV	eq	Π					
L'AIC	#	1	96	ی				i				Ţ	est h	Test Number	ב										-
											CONCENTRACTION	11.5	V.T.V												<del>-</del> -71
MALFORMATION	1	<u>u</u>	16 84 1.41	1.4	F	1	3.6	_	1		ואר ביו ביו ביו ביו ביו ביו ביו ביו ביו ביו		1	-	Ę.	F									
Severe	_	"	7.6	:5	2 !!	0	30'00	6 0,03	50,07	00	λοφ. 100	600	8,0	0,1	C1.5 7/4	- H 2 II								TOTAL	,
Stunted			T	İ	+	+	+	_	_	Ţ			+	$\dashv$	+	+	_	_							
Gut			T	$\dagger$	+	+	+	$\perp$				T	+	$\dashv$	+	_	$\dashv$	1				$\dashv$	+		
Edema			T	T	+	+	+	_	_			$\dagger$	+	+	+	+	_	$\perp$			$\exists$	$\dashv$	+		
Multiple			T	<del> </del>	$\frac{1}{1}$	+	+	+	1			Ť	1	-	+	$\dashv$	_	_			1	1	_		
Cardiac			T	T	<del> </del>	<u> </u>	<u> </u> _	<u> </u>	_			İ	+	$\frac{1}{1}$	+		$\perp$	1			$\dagger$	+			
Abdominal				$\dagger$	$\dagger$	-	+	-	_				$\dagger$	+	+	+	$\perp$	1			1	$\dashv$	-		
Facial			Ī	<del> </del>	+	<del> </del> -	<u> </u>	+	1			Ť	$\dagger$	+	$\frac{1}{1}$	_	$\frac{1}{1}$	_		T	T	+	<u> </u>		
Cephalic				<del> </del>	-	<u> </u> 	+	+	+			T	$\dagger$	$\frac{1}{1}$	+	+	_				Ť	$\dashv$			
Optic				<del> </del>	$\vdash$	+	+	$\dotplus$	_			1	$\dagger$	+	+	+	+	_			$\top$	$\dagger$	+		
Tall				<del> </del>	+	+	+	1	$\bot$			1	$\dagger$	$\frac{\perp}{1}$	+	-	+	_			T	+	+		
Notochord			Ī	$\dagger$	+	+	+	+	1			İ	$\dagger$	<u>-</u>  -	+	+	+	4			$\dashv$	+			
Fin				T	$\dagger$	<u> </u>	+	+	$\downarrow$			Ť	1	+	+	+	-	_	Ī		1	1			
Face			T	$\dagger$	+	+	+	+	_			1	+	+	+	$\dashv$	-	_			j	+	┪		1
Eye			T	†	$\dagger$	+	+	+	_			Ť	$\dagger$	+	+	+	+	_	I		1	+	+		
Brain					+	+	$\frac{\perp}{1}$	+	$\downarrow$			+	+	+	+	+	_	_				+			
Hemorrhage					$\dagger$	+	+	_	$\downarrow$			$\dagger$	$\dagger$	+	+	+	1	_		1		1	+		
Cardlac			T	$\dagger$	$\dagger$	+	+	$\perp$	1			$\dagger$	$\dagger$	+	+	+	<u> </u>	1		1	+	1			
Bilisters				$\dagger$	+	+	+	+				Ť		+	+	1	+			1	$\dashv$	+			
Other-specify			T	$\dagger$	$\dagger$	+	+	-			İ	1	+	+	+	$\dashv$	$\dashv$	_				+			
No. Malformed	0	0	8	1	"	7   5	01	7 26	75	2	77	2	12	-   -	<u>  </u>   _	#	-				Ì	-			
Total No.	3		50	12		1	17		7 2	3 3	\$ 2			7 6	- -	1	-	_		T		$\dashv$	+		Ī
Comments:										→1				200	1	$\dashv$	4	_					-		

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		°Ž	0	0	0	25		0	0	0	c	O	0	۵		4	∞	39	40					
			0	0	Ø	0		0	Ø	0	0	0	0	0	~	2	3	19	20		 		 	- 1
Į.		Total	٥	0	0	151		0	0	0	0	Ω	0	0	0	7	70	3	20					
2-AAF		96 hr															-				 		 	
aterial	umber	72 hr	-	,	-											_			1		 		 	
Test Material	Test Number	48 hr			-														<b>-</b> -	-	 		 -	
		24 hr					- ~					,									 	-	 	
		FETAX (ml)																						
1	112 9B	Stock (ml)																						
Investigator Fax	Date	Concentration	FETAX	FETAX	5.5 BAN	2500 6-AN		0.02	0,025	0,03	0,035	6.04	5000	80.0	60.0	560.0	0,1	571.0	51.0					

	FE'	TAX SUMN	ЛАRY	SHEET	•			
	1 6						Test No.	
Test Mate	rial T	E				Investi	gator Tor	+
Source	Aldrich	Biama				Lab	SA	
CAS No.		Lot	No.			Test St	art Date	
Composition	on/Purity					Test En	d Date	
Solvent		Co	nc.			Test Un	its (i.e., mg/ml)	malmL
		DAY 1	DA	Y 2	DAY	7 3	DAY 4	DAY 5
Stock	)H				<del></del>			
Contro	i							
Highes	t Conc.				<del></del>			
FETA	X CONTROL=			= MORTA: RECO			MALFOR	MATION ==
	ed or Malformed	X 100 = %						
Solvent Con			<u> </u>	: <u>80</u> x 10			<u>o: 86 x</u>	100 = <u>D</u> %
Control Len			Solva	nt Control	X = _		:X	100 =%
	oncentration to						11, 11	
I I I I I I I I I I I I I I I I I I I			(					
TEST MA	TERIAL/CO	OMPOUND	: RES	SULTS				
TEST	MORTAL	LITY	ALFO	RMATION		STAT	ISTICAL TEST	USED
NOEL								
LOEL					Ť			
LC <sub>50</sub>	0.49			EC50		0.0	51	
95% Confide	ence limits O	.48-0.5	O	95% Con			s 0.049-	-0.052
T	TEST TERATO	GENIC INDEX	(TI =	LC <sub>50</sub> /EC	50 )			
<del></del>								
POSITIVE	CONTROL	: 6 AMINO	NICO.	TINAMI	DE (	6-AN)	RESULTS	
CONCE	NTRATION		MORTA	LITY			MALFORMA	ATION
5.5 mg/l	•	<u>:</u>	X 10	0 =%			:X 100 =	%
2500 mg,	L	<b> </b> :_	X 10	0 =%	,		: X 100 =	%

												-		_	_		_						_	_	_	_	
				-	- -	-		<u>-</u>				<u> </u>		 	ļ	 											- - 
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			\$ N	ρ	Ó	-	, 2		0	0	0	0	0	0	٥	0	0	ŋ	0	-3	40	40				_	
			1	0	, ,		4		٥	0	0	0	0	0	٥	0	9		3	[V	સ	8					
			Total	0	0	C	7		Ð	٥	0	6	0	0	0	0	0	9	7	œ	2	20					
77	<u>1</u> - (		96 hr	-	-	-	-										<b>-</b> -										
			-		$\vdash$	-	-																				
Test Material	Test Number	130111111	72 hr																								
Test N	Test		48 hr																	-							
			-	_							-					-	-			1	-		-				
			24 hr													-				<u>-</u>	-	-		-			
			(ml)																								
			FETAX (																								
									$\dashv$		$\frac{1}{1}$	+	1	1	-		+	<del>- </del>	-	+	1	-		+			
	9	, , ,	Stack (ml)																								
7 40	21/11		Stoc																								
			u		ł	14.9	ZY.																				
Investigator			Concentration	TAX	XX	اه: اح	2500 6-AN		0.035	40.0	0,045	0.03	0,055	90'0	0.065	6.3	0,35	٢   ١		7 0		e					
Invest	Date		Conce			5,5	25		Ó	Ó	Ó	0	0	0	Ó	9	0	410	0	0	0	5					

# FETAX MALFORMATION DATA

Investigator			T		
Dete	_		I cst Material	K 5	
DAIG	11/12/92		Test Number		
MATEODIAATION			CONCENTRATION		
MACLORMATION	F F 512 2500 5,035 a01	0045 205 1,055 0,0k 0.06			TOTAL
Severe					TVIOI
Stunted					
Gut					
Edema					
Multiple					
Cardiac					
Abdominal					
Facial					
Cephalic					
Optic					
Tall					
Notochord					
Fla					,
Face					
Eye					
Brain					
Hemorrhage					
Cardiac					
Blisters					
Other-specify					
No. Malformed	20 419100	10 18 21 38 42	7.7		
Total No.	4	07 07 07 27	27 27		
Comments:			16/12/21		

FETAX SUMMARY SHEET										
							Test N	0.		
Test Mate	erial Cour	narin				Invest	igator T	; <del>+</del>		
Source	Sigme	·	SE	1R-A	rmy	Lab	SA	-		
CAS No.		Lot	No.		(	Test Si	tart Date			
Composition	on/Purity					Test Er	nd Date			
Solvent		Co	nc.			Test Ur	nits (i.e., mg	/mt) m/	51 L	
		DAY 1	DA	Y 2	DA	Y 3	DAY 4		DAY 5	
Stock	pH		<del> </del>					<del>-</del>		
Contro	ol									
Highes	st Cone.									
	AX CONTROL =			= MORT REC	ALITY			FORMA'		
	otal Number	X 100 = %			100 = _	~	<del> </del>	V 400	_ ~	
Solvent Con	ntrol				100 = _	% %	<u></u>	X 100 X 100		
Control Les	ngth mn	1	Solve	nt Contro			mm	_ ^ 100	<u> </u>	
Minimum C	oncentration to	Inhibit Growt	h (MCI			<del></del>				
TEST MATERIAL/COMPOUND : RESULTS										
TEST	MORTAL	ITY N	<b>AALFOI</b>	RMATIO	N	STAT	ISTICAL T	EST US	ED	
NOEL										
LOEL								1 1 1		
LC <sub>50</sub>	168	. 74	EC <sub>50</sub>				59.79			
95% Confide		54, 17- 1	84.16	95% C	onfider	ice Limit	1s 58,5	2-6	1.08	
1	TEST TERATOG	ENIC INDEX	(TI =	LC 50 /E	C <sub>50</sub> )				and the Company of th	
									1	
POSITIVE	CONTROL:	6 AMINO	NICO.	TINAM	IDE	(6-AN)	RESUL	TS		
CONCE	NTRATION		MORTA	LITY			MALFO	RMATIC	N	
5.5 mg/l					-					

X 100 =

X 100 =

2500 mg/L

2		Total No %					 ) )						1 7 1	1 0 1	3 3 6	9 -	20 20 40	20,20 40			 	
iceial (RUMARIM		72 hr 96 hr					 												 		 	
Test Material	Test Number	24 hr 48 hr																	 		 	
		FETAX (ml)		•																		
401	11/12/96	Stock (ml)			5	Q																
Investigator	Date	Concentration	FETAX	FETAX	6-4N Sis	6-AN 250	Shoo	0.05	0.055	90.0	290.0	0.07	0.075	260,0	0	7.0	0 , 2	0.28				

FETAX MALFORMATION DATA

Investigator		1										-	7	Test Material	-										Ī
Date			1.									1		10141		4	V A A	OIDWMDIO	1	2					
	7	98	اھ									ī	est N	Test Number	<u>,</u>										
			1	14-7						03	CONCENTRATION	NT.N	ATIC	Z											7
MALFORMATION	I	芷	5.5	3	9	Swo, 6	80.0 20.0	20.0	13.7		10	F0.	1-0	151.5	-	_				<b> </b>	-	-			
Severe					-	1	-	1	1				,	-	1		1				1	+	_	I O I VI	ا د
Stunted		İ	T	$\dagger$	$\frac{1}{1}$	<u> </u>	+		_				$\dagger$	<del> </del>	$\frac{1}{1}$	-			$\dagger$	+	$\frac{1}{1}$	$\dashv$	$\frac{1}{1}$		
Gut			T		-	+	+	1	_			Ť	+	+	+	_	_		1	+	$\dashv$	$\dashv$	_		
Edema			Ť		+	+	-	_	_			†	+	+	+	+	1		$\uparrow$	$\dashv$	$\dashv$	$\dashv$	<u> </u>		
Multiple		T	Ť	$\dagger$	+	+	+	1.					$\dagger$	+	+	<u> </u>	_		十	$\dashv$	+				
Cardiac			İ	<del> </del>	<u>!</u>	<u> </u>	<del> </del>	<u> </u>				İ	$\dot{\top}$	<del> </del> -	<del> </del>	+	1		Ť	$\dagger$	$^+$	-	-		
Abdominal		T	T	$\vdash$	+	<del> </del>	+	+			I		+	- -	+	$\downarrow$	1		Ť	1	_	$\dashv$	_		
Facial		<del> </del>	T	+	+	+	+	+	_	Ţ	I	Ì		+	+	$\downarrow$	1		1	+	+	+	<u> </u>		
Cephalic		Ì	Ť	İ	$^{+}$	<u> </u>	<u> </u>	+	1			Ì	$\frac{\perp}{1}$	<u>- -</u>	+	1	1		Ť	1	$\dashv$	$\dashv$	<u> </u>		
Optic		T	T	Ť	+-	+	+	1	1		I	<u> </u>	$\dagger$	+	+	$\frac{1}{2}$	1				+	1			1
Tall		<del> </del>	Ť	İ	+	+	+		_			j	$\frac{1}{1}$	-	+		1		+		+	_			1
Notochord		T	Ť	Ť	+	+	$\frac{1}{1}$	-	+			Ť	+	-	+	1	1		İ	$\dashv$		$\dashv$	1		
Fln		T	T	T		+	+	1	$\perp$	1			+	$\frac{\perp}{\parallel}$	+	$\perp$	1			$\dagger$	$\dashv$	$\dashv$	+		1
Face				$\dagger$	$\dagger$	+	+	1	1			1	$\dagger$	+	+	+	_		1	+	$\dashv$	+	-		
Eye		T	Ť	Ť	-	+	<u> </u>	+	<u> </u>			Ť	+	+	+	1	_		$\dagger$	+	+	$\dashv$	<u> </u>		
Brain			Ť	$\dagger$	+	+	+	-	1			Ť	$\dagger$	+	$\frac{1}{1}$	+	_		1	$\dashv$	1	$\dashv$	<u> </u>		1
Hemorrhage		T	T	T	+	+	+	_	1				+		+	+	_		$\top$	+		$\dashv$	+		
Cardlac				$\vdash$	<del> -</del>	+	+	-	1	$oxed{\Box}$			$\dagger$	+	+	-	1		Ť	十	+	$\dashv$	<u> </u>		-
Blisters			Γ		$\vdash$	<del> </del>		_	_	$oxed{\Box}$		T	<del> </del>	+	+	+	1		十	$\dagger$	+	+			
Other-specify			İ	$\dot{\parallel}$	$\vdash$	<u> </u>	+	1	1		I	İ		$\frac{\perp}{\parallel}$	+	+	1		Ť	+	$\dashv$	+	+		
No. Malformed	0	977 0		0		0	0	19	167	140	107	39	24	1,4	<u>  </u> 				- <u>i</u> -						
Total No.		2	કુ	0	<u> </u>	<del></del>		1				29		7 6	+	+	1		Ť	$\dagger$	+	+	+		
Comments:									#			-41		1/7	4	4					-			٠	

## Appendix B

Metabolic Activation System Test Kit Design

## Version 4 FETAX Microsome Kit

Contents: ☐ Aroclor 1254-induc	ed 🗆 Isoniaz	tid-induced D Phe	nobarbital-induced				
□ β- Naphthoflavone-	induced $\square$ Ph	enobarbital-β- Napl	nthoflavone-induced 1:1 mix				
☐ Aroclor 1254-induc	ed-Isoniazid-in	duced 1:1 mix					
$\square$ Phenobarbital, $\beta$ - N	aphthoflavone-	induced- Isoniazid-	induced 1:1 mix				
substance is mixed wit substance. The 1:1 me	I it means that and the second in the second in the second in the second ing to the Nash	a lot of microsomes microsomes from a ivities have been ad assay. Mixed micro	from a rat induced with one rat induced with another justed to the proper number of osomes offer the ability to cover				
Microsome Specific A	ctivity	Units	s/mg protein				
Protein Concentration of BioRad Protein Assay			g/ml				
performed by _		date	<del></del>				
Nash Units of microsomes in KitU/ml							
performed bydate  FETAX  Positive Controls Date Performed Technician							
Microsome Quality Control:  FETAX positive control for Aroclor-induced microsomes.  Experiment							
Experiment	% Mortality	% Malformation	]				
FETAX Controls							
4 mg/ml Cyclophosphamide 4 mg/ml Cyclophosphamide +							
Microsomes							
Cyclophosphamide with mortality at the end of the Technician performing	he 96 hrs.		es should result in 50%				

FETAX positive contro	ol for Isoniazid	-induced microsome	<u>s</u>
Experiment	% Mortality	% Malformation	
FETAX Controls			
4 mg/ml			
Acetyl hydrazide			_
4 mg/ml			
Acetyl hydrazide +			
Microsomes			J
	isoniazid-micro	somes should result	in 50% mortality at the end of
the 96 hrs.			
Technician performing	Assay	D	ate:
			niazid-induced (mixed)
			- Isoniazid-induced 1:1 mix
Experiment	% Mortality	% Malformation	
FETAX Controls	]		
4			
4 mg/ml			
Cyclophosphamide			
4 mg/ml			
Cyclophosphamide +			
Microsomes			
	· · · · · · · · · · · · · · · · · · ·	T	1
Experiment	% Mortality	% Malformation	
FETAX Controls			
			]
4 mg/ml			
Acetyl hydrazide			
4 mg/ml			
Acetyl hydrazide +			
Microsomes			
Cyclophosphamide with		-induced-microsome	es should result in 50%
mortality at the end of t			
	soniazid-micros	somes should result	in 50% mortality at the end of
the 96 hrs.	<b>A</b>		
Technician performing	Assay	Da	nte:

## Storage:

Box 1-Microsomes: These must be stored at -80°C. If such a freezer is not available remove the tubes from the box and freeze under liquid nitrogen. There is one tube for each day of the experiment. Discard any left over microsomes and do not refreeze.

Box 2-Generator, glucose 6-phosphate dehydrogenase, cyclophosphamide (Aroclor 1254 positive control) and acetyl hydrazide (isoniazid positive control). Store frozen at -20°C.

## Kit Design:

Each kit is designed to allow the contents to be directly pipeted into a 50 ml Erlenmeyer flask without further calculation. The total volume in the flask is 20 ml regardless whether glass or plastic dishes are being used. Generally, plastic dishes are used to help maintain sterility. However, autoclaved glass dishes may be used when the test material binds to plastic. Simply discard the remainder and thaw the contents of new tubes on each successive day of the test.

## Thawing instructions:

Bring tubes to room temperature and with gentle rocking motion of the wrist mix the contents of the tube. Freezing often causes a salting out effect thereby requiring that the tube contents be resuspended in solution. As soon as the last ice melts, place the tube in an ice bucket. Never shake or vortex a tube to mix contents, always stir or gently rock into solution. This is especially true for the microsomes. Frothing indicates denaturation of protein which means enzymatic function is lost.

Adding contents to the 50 ml Flask: Use an appropriate Pipetman for small quantities and use a disposable plastic Pipet for larger volumes.

- 1. If appropriate, add correct amount of cyclophosphamide to flask or acetyl hydrazide.
- 2. If appropriate, add test compound.
- 3. Calculate the amount of additional FETAX-AB needed to reach 20 ml. Add this amount and gently swirl to mix.
- 4. Add correct amount of Generator to flask.
- 5. Add correct amount of microsomal protein to flask.
- 6. Add correct amount of Glucose-6-Phosphate Dehydrogenase to flask.
- 7. The add 10 ml to each glass dish or 8 ml to each plastic dish as needed. Discard any remaining solution or use it for chemical analysis.

## **Kit Contents:**

Compo- nent	Amount to Add/day	day 0	day 1	day 2	day 3	Kit
	(μl )	Total Quantity (ml)	Total Quantity (ml)	Total Quantity (ml)	Total Quantity (ml)	Grand Total (ml)
Generator	192.5	6.3	6.3	6.3	6.3	25.2
Glucose-6- Phosphate dehydro- genase	62	2	2	2	2	8
Micro- somal Protein	191	6.3	6.3	6.3	6.3	25.2
Cyclophos- phamide	10,000	21	21	21	21	84
Acetyl hydrazide						

<sup>\*</sup> Each kit holds enough material for 32 Petri dishes that contain MAS. There will always be some material left over.

## Kit Supplies Materials to Prepare the Following Dishes:

Dishes in an experiment that do not contain MAS.

FETAX solution only - 4 dishes

FETAX-AB solution - 2 dishes

FETAX solution plus 4 mg/ml cyclophosphamide (part of positive control)-2 dishes.

Dishes in an experiment that contains MAS.

FETAX- generator- microsomal protein (negative control)- 2 dishes.

FETAX- generator- microsomal protein treated with carbon monoxide-dithionite- 4 mg/ml cyclophosphamide (negative control)- 2 dishes.

FETAX- generator- microsomal protein + LC50 of test compound (control for interaction between MAS and test compound)- 2 dishes.

FETAX- generator- microsomal protein + EC50 of test compound (control for interaction between MAS and test compound)- 2 dishes.

FETAX- 4 mg/ml cyclophosphamide- generator- microsomal protein (part of positive control for Aroclor-induced microsomes)- 2 dishes.

FETAX- generator- (negative control for generator- no microsomal)- 2 dishes.

## For different concentrations of the test material:

FETAX-generator-microsomal protein-test compound-2 dishes per test concentration (20 total).

Total dishes requiring MAS-32.

### DEPARTMENT OF THE ARMY



US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND 504 SCOTT STREET FORT DETRICK, MARYLAND 21702-5012

REPLY TO ATTENTION OF:

MCMR-RMI-S (70-1y)

4 Dec 02

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

- 1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for this Command. Request the limited distribution statement for the enclosed accession numbers be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.
- 2. Point of contact for this request is Ms. Kristin Morrow at DSN 343-7327 or by e-mail at Kristin.Morrow@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

PHYLIS M. RINEHART

Deputy Chief of Staff for Information Management

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